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PHILOSOPHICAL TRANSACTIONS.

I. *Additional Evidence of the Affinities of the Extinct Marsupial Quadruped* *Thylacoleo carnifex* (OWEN).

By Sir RICHARD OWEN, K.C.B., F.R.S., &c.

Received October 5,—Read November 25, 1886.

[PLATE 1.]

SINCE the first indication of a large extinct Carnivore by a tooth obtained by Major Sir T. L. MITCHELL in the cavern discovered by him in Wellington Valley, Australia,* fossil remains from that and other localities of the same Continent have been successively transmitted to me, which I have referred to the extinct genus and species *Thylacoleo carnifex*. Papers descriptive of these fossils have been admitted in the 'Philosophical Transactions,' and their value has been enhanced by the comments they have excited in the works of contemporary palæontologists.† These eminent authors received the support, in reference to objections to my conclusions, of the (then) Curator of the Australian Museum, Sydney, Mr. GERARD KREFFT, who, in his contribution to the 'Annals and Magazine of Natural History,' series 3, vol. 18, 1866, p. 148, records his opinion that "the famous marsupial Lion was not much more carnivorous than the Phalangers of the present time."

* 'Three Expeditions into the Interior of Eastern Australia,' 8vo., 1838, vol. 2, p. 359, plate 32, figs. 10, 11.

† FALCONER, Dr. HUGH, F.R.S.: 'Proceedings of the Geological Society of London' for March, 1857; 'Quarterly Journal' of the Society, June, 1862, p. 353; 'Palæontological Memoirs and Notes,' 8vo., 1868, vol. 2, p. 437. Professor BOYD DAWKINS, F.R.S.: 'Quarterly Journal of the Geological Society of London,' vol. 20, 1864, p. 412. Professor W. H. FLOWER, F.R.S., 'On the Affinities and probable Habits of the extinct Australian Marsupial *Thylacoleo carnifex*, OWEN'; 'Quarterly Journal of the Geological Society of London,' vol. 24, 1868, p. 307.

The species of carnivorous Phalanger is not named. No evidence of such by fossil specimens has reached me, nor have I found such exceptional habit of an existing species of *Phalangista* elsewhere noted.

As the palæontological survivors of Dr. FALCONER and Mr. KREFFT have not signified any opinion of the fossil evidences, more or less fragmentary, of *Thylacoleo* discovered subsequently to the papers above cited, I deem it due to them to make known the most complete and instructive example of the mandibular and dental structures of the mooted species which have yet reached me.

The subject of the annexed drawings (Plate 1) is the right "ramus" of the lower jaw, which was extricated in the present year (1886) from breccia of the Wellington Valley cave. A careful cast of this specimen has been transmitted to me by G. P. RAMSAY, F.L.S., successor to Mr. KREFFT, and present Keeper of the Australian Museum of Natural History, Sydney, New South Wales, together with the three drawings of the original specimen, natural size, herewith annexed.

The dentition of this specimen closely repeats the characters of the mandibular teeth described and figured in fragmentary specimens.* The additional characters, which I interpret as decisive of the carnassial nature of *Thylacoleo*, are those of the hinder end of the lower jaw, including the articular process. This part is a "condyle" transversely extended, antero-posteriorly convex, as in both Lion and Tiger. The angle, α , of the ramus is bent inwards as in other Marsupials, including the smaller existing pouched Carnivores. In *Thylacoleo*, to add to the force of the biting actions of the mandible, a subsidiary ridge ending in the process, figs. 1 and 3, b , is developed from the outer side of the broad angle of the jaw; the homologue of this ridge and process, wanting in placental *Carnivora*, is developed in the largest of the existing marsupial ones, e.g., *Thylacinus cynocephalus*. The coronoid process of the mandible in *Thylacoleo* (figs. 1-3, d) rises high above the condyle, and broadens antero-posteriorly as in the feline placental Carnivores. The entry of the dental canal is shown at e , fig. 2; the exit at f , fig. 1.

As the figures in Plate 1 are of the natural size, descriptive dimensions are omitted.

What to me is of most interest in this decisively instructive fossil are the evidences of carnivorous modifications superinduced upon the primitive, and at present prevailing, *diprotodont* marsupial type. In the mandible of the vegetarian kangaroo (*Macropus*) the incisive part of the dental series is represented, as in *Thylacoleo*, by a single pair of large incisors; but these, as in the allied genera *Dendrolagus*, *Bettongia*, *Hypsignathus*, &c. are prominent, depressed instead of compressed, having a smooth flattened surface, with lateral margins, instead of the sharp-pointed and hinder trenchant

of the incisor, which is granular (figs. 1 and 2, p. 4) in the case of the latter genus, as the granulated granular surface of the incisor of *Thylacoleo* is shown in which

as many vertical grooves on the side of the crown terminate. Moreover, the so modified premolar is followed in the vegetarian Diprotodonts by four broad-crowned bruising teeth, instead of the suddenly reduced couple of conical molars (*m*, 1 and 2, figs. 1 and 2, Plate 1) by which *Thylacoleo* resembles the placental Leonines. I view with interest the engrafting of a carnivorous modification upon a marsupial type of teeth and bone in a species equal as to size and force to grapple with and slay its ancient vegetarian contemporaries—the greater herbivorous Diprotodonts and Nototheriums, the large, now extinct, Kangaroos, *Sthenurus*, *Protemnodon*, and the huge extinct Wombats (*Phascolonus*)—types of pouched mammalian families, surpassing in bulk any of the allied still existing species.

The picture of mammalian life in the Australian continent paralleled, of old, that still manifested in Asia and Africa: huge herbivorous quadrupeds were kept in check by large and powerful carnivorous ones, but both were represented by species of a lower grade of organisation; and the inferior cerebral development of the *Marsupialia* may be taken into account when we attribute to the advent in Australia of the Bimanous race the extirpation of the beasts affording the greatest quantity of animal food, and the consequent reduction of the pouched families to such smaller existing species as are still able to escape by concealment in burrows, trees, and brush forests.

ADDENDUM.

(Added 22nd December, 1886.)

Since communicating the foregoing Paper, I have received from GEORGE FREDERIC BENNETT, Esq., Corresponding Member of the London Zoological Society, a large portion of a mandible of *Thylacoleo carnifex*, discovered in the post-pliocene bed of King's Creek, Toowoomba, Queensland, Australia; it is in the same semi-fossilised condition as the Diprotodont remains from that locality.

The specimen may be seen, together with the cast of the entire mandibular ramus from the Wellington Valley Cave, New South Wales, in the Geological Department of the British Museum of Natural History, Cromwell Road.

R. O.

DESCRIPTION OF THE PLATE.

- Fig. 1. Mandible of *Thylacoleo carnifex*, nat. size, outside view.
- Fig. 2. Mandible of *Thylacoleo carnifex*, nat. size, inside view.
- Fig. 3. Hind end of mandibular ramus of *Thylacoleo carnifex*, nat. size.

(References to parts in these figures are explained in the text.)

II. *Remarks on the Cloaca and on the Copulatory Organs of the Amniota.*

By HANS GADOW, *Ph.D., M.A., Lecturer on the advanced Morphology of Vertebrata, and Strickland Curator in the University of Cambridge.*

Communicated by Professor M. FOSTER, Sec. R. S.

Received March 11,—Read March 25, 1886.

[PLATES 2-5.]

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A RICH material of Crocodiles and Alligators has enabled me to clear up several points in the structure of their sexual apparatus, which have hitherto escaped notice, probably owing to the scarcity of suitable specimens. This latter difficulty has been removed in my case by the liberality of the University of Cambridge and of the Royal Society, which has enabled me, in conjunction with Dr. GLASELL, to

construct a special house to keep alive, and in a healthy condition, a considerable number of Reptiles of all orders.

The study of fresh material possesses great advantages over that of preserved and diseased specimens, especially though if the anatomical results can be checked by observation of the functions of the organs.

For a specimen of *Siphonops annulatus* I am indebted to Dr. A. GUNTHER, for some of the Alligator embryos to Professor W. K. PARKER. Struthio embryos had been collected by Mr. SEDGWICK, aided by a grant from the Royal Society. Several well-preserved *Ornithorhynchus* and *Echidna* Mr. SEDGWICK kindly put at my disposal. Adult specimens of *Struthio*, *Rhea*, *Casuarus*, and *Apteryx* belonged to the museum of the University of Cambridge, and came for the most part from the Zoological Garden, in London. In connexion with these investigations turned up several questions concerning the Cloaca, which suggested a comparative treatment of the whole cloacal region and of the copulatory organs throughout the Amniota.

Of Reptiles the following material was at my disposal :—

- **Alligator mississippiensis*. Several embryos and several fresh specimens of about 5 feet in length.
 - Crocodylus palustris*. Several baby specimens.
 - Crocodylus acutus*. About 3 feet long.
 - **Crocodylus biporcatus*. Numerous fresh specimens from 8 inches to 24 inches in length. One specimen of about 3 feet.
 - Crocodylus vulgaris*. Male copulatory organ of adult; specimen in R.C.S.
 - Crocodylus* sp.? Half adult female; preparation in the Cambridge Museum.
 - Monitor indicus*.
 - **Lacerta ocellata*. Numerous specimens.
 - **Lacerta viridis*. Numerous specimens.
 - Psammosaurus scincus*.
 - **Hatteria punctata*. Male and female.
 - **Tropidonotus natrix*. Numerous specimens.
 - Pelophilus madagascariensis*. Male and female.
 - **Testudo græca*.
 - **Emys europæa*.
 - **Clemmys caspica*.
- } Numerous specimens.

There is an abundant literature on this subject, but the descriptions and conclusions found in it frequently agree neither with each other nor with the actual facts. A discussing review of the literature would lie beyond the scope of this paper. I append, however, a list of the papers which I have consulted.

* The species marked with an asterisk were, or are, kept in the Reptile-house.

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Of text-books, see BALFOUR, BRONN-HOFFMANN, CUVIER, GEGENBAUR, HUXLEY, NECKEL, MILNE EDWARDS, STANNIUS, TIEDEMANN, and WIEDERSHEIM.

I. *Muscles of the Cloaca.*

Crocodylia (figs. 3, 5, 8, 9).—After removal of the skin appears on each side of the anal opening a continuation of the *M. rectus abdominis lateralis*, which, a little beyond the caudal corner of the opening, is attached to the *M. ilio-ischio-caudalis*, the chief mass of the lateri-ventral muscles of the tail.

Partly covered and crossed by the *M. rectus lateralis* is the *M. transversus perinei*, which, enclosed between the *rectus lateralis* and the ischiadic portion of the lateral tail muscle, arises from the first processus transversi of the caudal vertebræ as a thin semi-aponeurotic plate, gradually thickens, and is inserted with transversely running

fibres on to the caudal end of the Symphysis ischii and to the neighbouring corner of the anal opening. Contraction of this muscle opens the anus.

The side walls of the anal portion of the cloaca are surrounded by a rather thin layer of muscles, which arise in the median line between the right and left halves of the M. ischio-caudalis, from some of the first chevron bones of the caudal vertebrae. Their fibres are directed more or less transversely towards the margin of the anus, where they are attached. The most superficial portion of this system has, however, a more longitudinal direction and is somewhat differentiated off from the rest; its fibres meet at the anterior corner of the anus and surround the latter like a sphincter. Their contraction closes and elongates the anal slit, whilst through the action of the more transverse, deeper mass the anal walls are drawn inwards and are thus likewise closed.

All these muscles, viz., the M. transversus perinei, s. transversus lateralis, s. levator, and the M. transversus medianus, s. sphincter, together with the M. rectus lateralis, are supplied by a strong branch of the ischiadic plexus. In *Alligator mississippiensis* this nerve-branch comes from the stem α , i.e., the first postsacral spinal nerve. It leaves the pelvis laterally from the tendinous insertion of the M. ischio-caudalis, between this tendon and the posterior margin of the ischium near the symphysis, and then it supplies, situated superficially, the muscles, sending out at the same time cutaneous branches.

Considering the nerve-supply, it seems more reasonable to suppose that the sphincter muscles are, at least in the Crocodilia, a differentiation of the post-pelvic portion of the system of the M. rectus abdominis, and not of the true caudal muscles.

In the *Chelonia* the penis is not attached to the pelvis, nor is it in connexion with any anal muscles; but to its dorsum, not far from the glans, are attached a pair of long retractor muscles, which pass laterally from the M. pubi-caudalis (fig. 10) (flexor caudæ obturatorius, BOJANUS) into the pelvis, and arise from one of the most anterior lumbar vertebrae. The position and nerve-supply of these peculiar muscles show that they are differentiated from the MM. lumbo-caudales (flex. caudæ lumbales, BOJANUS). There is consequently no homology whatever between this Chelonian retractor (sometimes called M. ischio-cavernosus) and any of the Avian and Mammalian copulatory muscles. Its function is somewhat complicated. When the penis is erected and protruded, the muscle acts as the retractor, but when the organ is in a relaxed condition and, when at rest, doubled up and withdrawn into the cloaca, as shown in fig. 26, contraction of the muscle, owing to its pulley-like course round the M. pubi-caudalis, undoubles it and protrudes it so far that the glans becomes visible in the anal opening. This is seen in micturating Tortoises, because the penis has to be straightened in order to withdraw the glans from its position ventrally from the fold *p*, where it blocks the opening of the bladder. During defæcation, on the other hand, the doubled halves of the penis are pressed against each other, and, together with the fold *p*, which is likewise pressed down, effectively protect the urino-genital

apparatus. This explains why in the Tortoises defæcation and micturition are separate acts. How the penis becomes doubled up is not very easily seen; probably through the greater elasticity of its grooved side and through the contraction of the walls of the cloacal chamber.

The vestibulum cloacæ is capable of considerable shortening through the longitudinal unstriped muscles of its walls and through the paired *M. pubi-caudalis*, which—arising from the transverse processes of some of the caudal vertebræ near the anal opening, and being attached to the splanchnic side of the pubic symphysis—can by its contraction bend the tail; at the same time both muscle-bands exert a pressure upon the lateri-ventral walls of the outer cloacal chamber. Anal muscles proper, like a distinct sphincter, are not developed in the Chelonia. The *M. ischiocaudalis* (*flexor caudæ ischiadicus* of BOJANUS) arises from most of the postsacral and caudal vertebræ; its fibres run transversely, and form a broad, although thin, muscular layer, which, besides on the tuber ischii, is inserted aponeurotically on the visceral side of the ischiadic symphysis. From the caudal corner of this symphysis to the anus the muscle-fibres meet with those of the other side, and being partly attached to the cutaneous lips of the anus, bridge over the whole cloaca. They act upon the latter as a constrictor. Some of the most superficial fibres, between the caudal margin of the ischium and the anterior corner of the anus, assume a more longitudinal direction and an independent aspect; they are described by BOJANUS as *dilatator cloacæ*, whilst in *Testudo græca* they are hardly developed.

In the *Birds* (fig. 15) a typical *M. sphincter ani* is formed from the same muscular layer just described in the Chelonia. This layer is differentiated into two muscles: (1) *M. transverso-analis*; it arises in a variable way from the distal caudal portions of the pelvic bones: in rare cases also from the transverse processes of some caudal vertebræ; its fibres form in the medio-ventral line a more or less aponeurotic junction with those from the other side, and are also attached to the anterior margin of the anal opening, where they are blended with (2) the *M. sphincter*. The fibres of the latter surround the anal opening in the typical way, and have no connexion with skeletal parts, except indirectly where there are a few muscular slips attached to the flexor muscles of the tail or to the skin in which the tail feathers are lodged. The *M. sphincter* and the *M. transverso-analis* are, like the Cloaca, supplied by nerves from the plexus pudendus. In *Rhea* (fig. 15) there is on each side a double *M. levator ani*, which arises from the distal portions of the pelvic bones, and is attached to the dorsal corner of the anus. It is probably a differentiation of the *M. transverso-analis*. We can derive the Avian arrangement easily from that of the Chelonia if we imagine that in the Birds the *M. ischio-caudalis* of the Chelonia has lost its origin from the tail, and that a separation took place between the anal portion (future *M. sphincter*) and the portion between the anus and the pelvic bones. The rest of the reptilian ventri-lateral muscles between tail and pelvis is represented in the Birds by the *MM. pubi- and ileo-coccygei*.

In those *Carinatae* which, like the *Anatidae*, possess a copulatory organ, the latter is placed slightly asymmetrically on the left side of the ventri-lateral cloacal wall. In connexion with its basal portion are several muscular bundles, which somehow seem to be able to protrude and to retract the penis. They are merely branches from the inner portion of the voluntary sphincter ani, which itself in its ventral half is more developed towards the left side. It seems probable that through the action of the inner portion of the M. sphincter the vestibular walls can be everted, and that with them the penis is protruded.

In the *Ratitae* the strongly-developed copulatory organ necessitates the presence of special copulatory muscles. There is in *Rhea* on each side a M. protractor penis, which in a similar way as in *Anas* is developed from an inner layer of the sphincter, but it goes as a rather independent muscle to the lateral portion of the basis penis. In *Struthio* it receives a slip from the M. transverso-analis, which descends from the pelvic bones. A pair of retractores penis arises from the pelvis, and is attached to the ventral aspect of the middle portion of the organ.

The copulatory muscles of the *Carinatae* are consequently derived from the M. sphincter ani solely, whilst in the *Ratitae* they are partly also differentiations of muscles, which are still attached to the pelvis, and are therefore skeleto-genital.

Saurii.—The numerous anal and copulatory muscles of the *Lacertilia* have been described and figured in the 'Morphologisches Jahrbuch,' vol. 7. The striped retractor muscles of the two penes cannot be compared to any of the other *Sauropoda*, although they are likewise differentiations either of the ventral caudal muscles or of the M. sphincter. The latter is almost independent, arising, however, from some of the first transverse processes of the tail vertebræ. *Hatteria* possesses a M. perinei a. transverso-analis like the Lizards; also a M. transversus medianus almost exactly like that described in the *Crocodilia*; its most superficial fibres are transformed into a distinct outermost sphincter, which, according to the shape of the anal opening, lies transversely and not longitudinally as in the *Crocodilia*.

From the outside of the M. transversus medianus, and covered by the M. sphincter, starts on each side a broad but thin muscle, which runs back and attaches itself aponeurotically on the skin and on the fascia of the ischio-caudalis, behind the anus. It resembles a very similar pair of muscles of the Lizards.

The odoriferous glands receive a coating of striped muscular fibres from the M. transversus medianus.

The anal and copulatory muscles of the *Mammalia* show the greatest differentiation.

In the *Monotremata* a broad, striated muscle arises from the ischium and attaches itself with a small portion to the root of the crura penis, sending at the same time fibres along the dorsum of that organ; it is a M. ischio-cavernosus; its greater portion, however, runs along the ventral and lateral walls of the cloaca, attaches itself to them, and extends as far as the sphincter ani, with which its fibres partly blend. Through the action of this muscle undoubtedly the cloaca can be considerably

shortened, and, since the penis is not attached directly (except through the ischio-cavernosus) to the pelvis, the organ can be protruded. The sphincter cloacæ externus is strong, and an inner portion of it shows the division into a sphincter ani and a sphincter of the opening for the penis. Retraction of the copulatory organ is perfected (besides by the relaxation of the *M. ischio-cloacalis* just described, and by contraction of the pelvic portion of the ischio-cavernosus) by the assistance of a small non-striped muscle, running along the dorsum to the glans penis, and forming the continuation of the longitudinal muscular layer of the sinus uro-genitalis. During the erection this small muscle acts as *M. levator penis*, with which it is homologous in other Mammalia.

The sphincter ani consequently does not take a share in the muscle-supply of the copulatory organ, and thus exhibits a difference from the Birds and Lizards.

All other Marsupial and Placental Mammalia possess likewise a muscle which arises from the pubischium and which grasps the crura penis; it may, however, arise from the ischium or from the pubis only (*M. ischio-cavernosus* s. *erector penis*).

The sphincter ani externus and the *M. bulbo-cavernosus* are to be looked upon as originally one mass, divided, hand-in-hand with the disappearance of the cloaca, into a dorsal portion or anal sphincter, and into a ventral portion or *M. bulbo-cavernosus*, s. *accelerator urinæ* (constrictor vestibuli in the female), with a third portion, the *M. urethralis*. The *M. levator ani*, the *M. transversus perinei*, and the *M. ischio-cavernosus* remind us of the Ratite arrangement. A *M. levator penis* is found in most Mammals, and is fixed to the dorsum penis. Sometimes, e.g., in *Chiromys* and in the Marsupials, it arises from the crura penis or from their fascia, and, therefore, still retains Monotreme conditions. In most Placentalia it has become attached to the skeleton, arising from the symphysis pubis (Rodents, Elephants) or from the fascia below it (Monkeys), from the ischial tuberosity (Hedge-hog), from the pubis and ischium with a broad origin (Horse), or, lastly, from the first caudal vertebrae, in which case it passes to the right and left from the rectum (Carnivora). In Man it is much reduced. Its chief action is frequently only the compression of the *venæ dorsales penis*.

Attached to the urethral side of the penis, sometimes extending down to its glans, is another, often very powerful, likewise non-striped muscle, the *retractor penis*, fig. 28. It is best developed in those Mammals in which the copulatory organ is lodged in a canal formed by the abdominal skin, but it is absent in the Rodents and Carnivora, and in those Mammals which have a free or pendent copulatory organ (Primates, Chiroptera).

It arises either from the sacrum or coccyx (Marsupials, Horse, Boar), and passes in this case on each side of the anus, or it comes from the ventral region of the sphincter ani (Cetacea, Ruminants); in the Bull it attains a length of about 30 cms., and each of its halves is more than 1 cm. in thickness.

Distinctly copulatory muscles in the Mammalia are consequently derived from

skeletal and also from non-striped muscles. How such involuntary muscles can be separated off from the longitudinal muscularis of the intestinal canal is shown, besides by the *M. levator* of *Ornithorhynchus*, by the *Crocodylia*. There is in *Alligator* (figs. 3–5) a broad, rather double band of non-striped muscle, which, near the place where the ureters enter into the cloacal wall, leaves the latter, perforates the voluntary *M. transversus cloacæ*, and firmly attaches itself to the fascia which covers the *M. ischio-caudalis*, rather near the medio-ventral line. There can be little doubt that this muscle is an additional protractor or shortener of the cloacal chamber, and helps to bring the copulatory organ to the surface.

II. *The Nerve-Supply of the Cloacal Region.*

The lumbar and sacral plexus of the Reptiles has been described and figured in the 'Morphologisches Jahrbuch,' vol. 7, but without reference to the plexus pudendus proper.

In *Alligator mississippiensis* (fig. 3) the primary sacral nerve δ is as a rule the twenty-sixth spinal. The obturator nerve is composed of $\alpha + b$, whilst the ischiadic plexus is formed by the greater portion of α , the entire stem δ , and a considerable part of α , which is the first post-sacral nerve. α sends out the following branches:

1. Several strong branches to the powerful *M. caudi-femoralis*, which receives further on a similar supply from β and γ .
2. A long nerve which passes to (cf. p. 10) the caudal margin of the symphysis ischium, and then supplies a portion of the *M. rectus lateralis*, the *M. transversus lateralis s. medianus*, i.e., the striped muscles of the anus.
3. Several entirely cutaneous branches are distributed over that region.

β and γ supply, like the other caudal spinal nerves, the *M. caudi-ischiadicus*; the share of β in this is, however, very small.

The stem β receives a branch from γ and from α , through which combination a sort of individually most variable plexus is formed; it supplies with several branches the muscles of the dorsal and of the lateral wall of the urinary chamber, and, moreover, the penis or clitoris, and lastly through many ramifications the rest of the whole cloacal vestibulum s. proctodæum.

The sympathetic system is arranged as follows:—In the lumbo-sacral region down to α it is very regularly composed of metameric ganglia, rami communicantes, &c.; the peripheral branches supply the testes, kidneys, blood-vessels and the gut; no changes regarding the nervous system are visible at the level of ϵ , the fifth prosacral nerve, at about which level the ileum passes into the rectum.

A remarkable change, however, takes place at α . The usual ganglion is somewhat removed from the stem on to the lateral chain, and numerous nerve branches are sent to the intestinal wall. In level of β all this is changed. The sympathetic system is reduced to a paired chain, composed of very thin rami communicantes sent from β , γ , δ , &c. There are no ganglia visible, and the chain supplies merely the caudal

vessels. No branches are sent to the cloaca, the anus, or to the copulatory organ. This break between an essentially vasomotor and an ordinary visceral supply taking place between α and β coincides fairly well with the end of the rectum and the beginning of the interior cloacal chamber *s. urodæum*.

Microscopic examination of osmic preparations of the various nerves mentioned above showed the following:—

Nerves supplying the *M. caudi-ischiadicus*.—The ordinary somatic structure.

Nerves of stem β , supplying the rectal wall.—Consisting of two portions, one non-medullated, the other with fine medullated fibres.

Nerves to the penis.—Many non-medullated (“sympathetic” vaso-motor), and many fine medullated (visceral) fibres; the whole nerve agreeing exactly with a typical *N. erigens* in structure.

A piece of nerve of the lateral sympathetic chain between γ and δ agrees much with the rectal branches from β ; it is a visceral nerve with small medullated fibres, and a large amount of non-medullated fibres.

In the Lizards the anal and sexual muscles are likewise supplied by postsacral nerves, which chiefly belong—like in the Crocodiles—to stem α in *Monitor*, *Cnemidophorus*, *Hatteria*; or to stems $\alpha+S$ in *Cyclodus*, *Platydictylus*, *Lacerta viridis*, *Ophryocessa*, *Polychrus*; or to stems $\alpha+\beta$ in *Chamaeleon*.

The cloacal region in *Emys* is supplied chiefly by the 22nd+23rd+24th nerves, *i.e.*, $\gamma+\delta+\epsilon$, the penis by the 24th and 25th= $\epsilon+\zeta$. The same formula applies to *Testudo graeca*. All these cloacal and copulatory nerve-branches run between the cloacal lateral walls and the *M. lumbo-caudalis* and *M. pubi-caudalis*, branching off from the whole spinal nerve-stems near the middle line.

III. The Modifications of the Cloaca.

The internal or cephalic end of the cloaca of the *Crocodylia* is marked by a very prominent ring-wall (fig. 23, *rc*), which is produced by the concentration of the circular muscular fibres. Somewhat half-way between the cephalic end and the anal opening is another fold (fig. 22, *F*), chiefly formed by the submucosa of the cloacal walls, most prominent on the dorsal side. These two semi-lunar folds are frequently so high and well developed that their opposite lips touch each other. They divide the whole cloaca into two chambers. The anterior, inner or cephalic, one has the same coatings as the rectum, but its inner walls are smooth and different in structure from the rectum. Into the dorso-lateral sides the ureters open separately, just above a prominent papilla. This chamber is either empty, or filled with the clear, almost colourless urine, which can distend this “urinary chamber” into an oval shape of very large size. It never contains fæces, which only pass through it. Such a chamber is peculiar to the *Crocodylia*. At the first glance we should compare it either to the chamber UD, or, because of its shape and partial function, to the chamber CD of

Saurians and Birds, but in neither case would the folds bordering its cephalic and caudal ends correspond with those of the other Sauropoda. In fact the urinary compartment described above is homologous with the chambers UD and CD of the Saurians, Snakes, and Birds. This I am able to prove by the condition of things prevailing in very young, but already hatched, Crocodilia (fig. 23). In *Alligator mississippiensis* (snout to anus 13 cms.) the ureters open into a small roundish chamber, which is bordered head- and tailwards by a high and very prominent circular fold. The fold towards the head is situated closely above the urinary orifices, and leads into a slightly larger chamber, CD, the inner walls of which are very irregular through high longitudinal and oblique folds; the lumen of this chamber CD is small, and shows the same internal structure as the rectum above, from which it is separated by another prominent and very thick fold. Externally both chambers CD and UD are surrounded by a powerful layer of chiefly circular muscles, which mark the termination of the rectum very distinctly, and give the chambers UD and CD the external appearance of one swollen bulb. The inside of both chambers shows numerous folds, finer and more villous in UD. In young *Crocodylus palustris* (snout to anus 11 cms.) a very similar condition prevailed, but the inside of chamber UD was smooth, velvet-like, and remarkably different from that of CD, the mucosa of which showed the same structure as the upper rectum. In young *Crocodylus biporcatus* (snout to anus 15 cms.) a remarkable difference is observed. The fold *rc* has almost entirely disappeared, and the chamber CD, besides now forming one compartment with UD, is considerably elongated; the inside shows only a few longitudinal folds, the walls of the two united chambers are thin, and much of the former strong muscular coating has given way to thin longitudinal fibres.

In still older specimens of *Crocodylus* and *Alligator* the original two chambers are transformed into one thin-walled, much-distended, and inside almost smooth compartment, shut off from the rest of the rectum by a sphincter and fold like that of the adult. This shows that the peculiar arrangement of the adult Crocodilia is secondarily acquired, and that part of the rectum, viz., CD, is transformed together with UD into a room intended for the exclusive retention of urine. The outer, posterior or extra-pelvic chamber (vestibulum) is characterised by a much stronger development of the longitudinal muscles, a considerable portion of which goes as a detached band to the post-anal region of the tail (cf. p. 14); its dorsal wall is much longer than the ventral, like in all animals with a longitudinal anal opening. Near its lateral margin opens on each side a complicated musk-gland at the fundus of a deep recessus. The walls of the latter can, like those of the gular musk-glands, be everted at will like the finger of a glove, chiefly through the pressure of the superimposed *M. sphincter transversus*. The sneary contents of the gland-bags are pressed out by the non-striped muscular coating of the bags, from which runs a likewise non-striped cord to the *cura penis*. It is generally supposed that through these strong scent-glands the sexes are enabled to find each other, but besides this sexual advantage they seem to be used as warning

organs, an idea which is suggested by the fact that all these glands secrete already in very young specimens, and that the vicious little Crocodiles (from one to two feet in length) kept in my reptile-house evert them when very angry. On the ventral wall, and immediately towards the outer side of the fold between the inner and outer chamber, is situated the penis. Its epithelial coating is continuous with that of the fold. The organ itself is attached to the caudal corner of the ischiadic symphysis by a strong and roundish fibrous band (figs. 1 and 8), which arises single from the ventral sides and forms partly the continuation of the two fibrous halves of the penis; the bulk of the crura penis (comparable to the corpora cavernosa) is not attached to the pelvis, as generally stated, but projects backwards towards and into the pelvis.

This portion of the crura penis is decidedly rich in lacunæ and other venous cavernosities, and is in all probability able to be swelled.

In the corner between the pelvis and the lateral side of each crus is a recessus, lined by the peritoneum. The fundus of this recessus is open and leads through a canal into the cloaca. The outer orifice of these peritoneal canals is protected by a small papilla. In the neighbourhood of these papillæ are several small blind sacs or lacunæ (cf. fig. 1), and further towards the glans I observed in the adult specimen of *Crocodilus niloticus* three or four soft papilla-like projections of the outer coating of this organ. They are furnished with sensory hedonic corpuscles.

The deep groove on the dorsal side of the penis ends towards the crura in a blind sac, into the further corner of which open the vasa deferentia.

In young female specimens, up to a total length of 3-4 feet, the clitoris is nearly of the same size as the male organ, but in larger specimens it is considerably smaller (cf. fig. 2). The whole structure of the organ is the same in both sexes, with the exception of the position of the openings of the vasa deferentia and the oviducts. The latter do not open into the recessus of the dorsal groove but on the brim, or rather outside, the intracloacal fold, close to the dorsal base of the clitoris. In both sexes, therefore, the genital tubes, although at first running along and piercing through (in the male) the dorsal cloacal wall, open in a decidedly ventral position and thus represent an arrangement similar to that of the Chelonia and Mammalia, whilst in the Lizards, Snakes, and Birds these tubes retain their original dorsal position. Moreover the whole cloaca of the adult Crocodilia is divided into a genital or copulatory and into a strictly urinal chamber, the latter being situated between the former and the rectum. As this also is an arrangement not found in other Vertebrata, it will perhaps not be unnecessary to make some remarks on the cloacal region of the other Sauropida and the Mammalia, especially because, in spite of BUDGE's first-rate monograph, we shall observe certain anatomical and physiological points which hitherto have escaped notice.

The *Lizards* represent a peculiar type (figs. 17 and 18). The transverse anal opening leads into a not very capacious cloaca, which in *Lacerta* is divided into an outer or more ventral and into an internal or more dorsal chamber. This division is formed

by two very large triangular flaps, one on each side, which arise from the inner, or median, root of each penis, and extend towards the medio-ventral line to the ventral or anterior margin of the anus. Each flap, more or less horizontally, lies inside the anal sphincter, so that, if the latter is closed, it is hidden from view. In the vestibulum thus formed open the penes and the neighbouring anal glands.

The slit between the free margins of the two flaps leads into a somewhat larger chamber, which is shut off from the rectum by a strong more or less circular fold. This fold is, however, very low on the ventral, but very high and thick on the dorsal wall. Thus is formed a rather deep dorsal recessus, into which open the urino-genital canals. In *Monitor*, *Lacerta*, *Anguis*, *Calotes*, the ureter and the vas deferens of each side are united into one short canal, which opens on a small papilla; in the female the two oviducts and the two ureters have four separate openings. In the genus *Lophura* both ureters unite, form a small pseudo-bladderlike dilatation, and open on one papilla in the dorso-median line; the oviducts have likewise one outer opening only, situated a little nearer towards the pelvis than the urinary opening, but they are divided by a longitudinal septum, which extends almost to their orifice.

The urino-genital recessus is surrounded by a thick and low nearly circular fold, formed entirely by the dorsal wall; it can close the recessus almost completely. This fold is arranged in such a way that, when pressed upon by the faeces coming from the rectum, the recessus is completely protected, but otherwise it leads the urine towards the urinary bladder, or, if that organ be not developed, into the rectal chamber.

This rectal chamber is very capacious and is marked off both against the rest of the rectum and against the cloaca by high and strong semicircular folds. Its internal structure in *L. ocellata* and *L. viridis* agrees with that of the rest of the rectum (which can sometimes form another pouch-like dilatation), but in *Monitor* its quite smooth and thin walls, with a very feebly-developed layer of circular muscle-fibres, bear more resemblance to the cloaca than to the rectum.

Most Lizards, with the exception of the *Monitors*, *Amphisbænidæ*, and some *Agamidæ*, possess a true urinary bladder; it is often of considerable size, and opens by a narrow tube on the ventral side exactly on (or slightly analwards from) the fold between the lower rectal and the urino-genital chamber. This position explains how, if the anal opening be firmly closed and the whole vestibulum be compressed, the urine can enter this bladder; and, secondly, how, by contraction of the bladder, part of the urine can enter the rectal chamber and there mix with the faeces, which, as is well known, almost invariably contain portions of the whitish-yellow urea. This rectal pouch is, therefore, a true cloaca in the physiological sense.

In the *Monitors* the inner divisions between the vestibulum and the urino-genital chamber are not well marked, and it is only by artificial means that folds corresponding to those of the *Lacertæ* can be traced.

The *Ophidian* type (fig. 19) is similar to that of the Saurians. The rectum forms a capacious, thin-walled, and smooth chamber, which can be shut against the rest of

the rectum by a thin but high circular fold, and against the cloaca by a high and thick semi-lunar fold coming down from the dorsal wall. The cloaca forms a dorsal recessus, into which open the oviducts; this sometimes common ostium is provided with a strong sphincter, which is in connexion with the fold just described. The ureters open side by side in one common slight niche on the dorsal side, towards the caudal margin of the anus; the small lips of the niche can close this little chamber. In the male snakes the ureters and vasa deferentia of each side are commonly united. A peculiarity of the *Ophidia* is, therefore, the separate and independent position of the oviductal orifices. The whole cloaca is, although imperfectly only, divided by horizontal or oblique dorso-lateral folds into a dorso-internal or urino-genital and a ventri-external or fæcal chamber—an arrangement similar to that of the Lizards. The latter chamber represents, of course, the vestibulum; it receives in its posterior dorsal wall the penes, and on the lateral side of them the well-developed anal glands. In the female the representatives of the penes are frequently very small, and reduced to mere shallow invaginations of the postanal wall, but the anal glands are developed much stronger than in the male, and fill up the whole space otherwise occupied by the male organ. Like in the Lizards, only still more completely, the rectal chamber retains both fæces and urine, acting therefore as a cloaca.

The cloacal arrangement of *Hatteria* (figs. 12 and 13) represents a type by itself, which, however, bears resemblance to that of the Lizards.

The transverse anal slit is bordered by non-prominent lips, and leads into a rather deep triangular chamber, which is lined by the continuation of the invaginated outer skin. In this chamber are seen three deep holes, viz., in each of the outer corners the openings of the anal glands, which have been accurately described by Dr. GUNTHER. They are of double the size of a common pea, and in the living animal have a strong, rather agreeable smell of musk and violets; the middle hole is round, and can be completely closed through the contraction of the *M. transversus medianus*. Its walls seem to possess thick lips; when cut open there is, however, only a very thin, although high, fold, which passes gradually into the lining of the cloaca, and contains no muscles. This fold is not clearly represented in any other reptile, although indications of it exist in many Lizards. The total absence of copulatory organs in *Hatteria* suggests that during copulation this circular fold can be protruded by inward pressure of the cloaca in order to secure conception. It would then bear a striking resemblance to the arrangement found in the *Cœcilia* (cf. p. 27). This hole leads into another chamber which in a half-grown female was 7 mms. long; its walls are lined with mucous membrane thrown into slight longitudinal folds. Inwards, towards the pelvic end, this chamber is bordered by another fold; this is circular, thick at its base, thinner at its free margin, and towards the dorso-median line it is raised into a triangular or conical flap, which is about 5 mms. high, and fits into the opening of the bladder in the opposite ventral wall. This fold corresponds to the fold F of other Reptiles. The next chamber is wider and longer and of the same

structure as the previous one, but with softer walls. Into it opens in the medio ventral line the long-necked bladder, and near the dorso-median line open on each side, near the base of a papilla, the ureters and the genital ducts. In the female there is one opening only for the oviduct and for the ureter of each side. This urodæum, or urino-genital chamber, is as usual shut off from the rectum by a high circular fold. Peritoneal canals are indicated by two recessus of the body cavity, which laterally, from the urino-genital orifices, extend into the cloacal wall, and below the fold F end each in a small non-perforated papilla. The latter were best developed in the male, and are represented in fig. 12.

The *Avian* type (figs. 14, 15, 16, 20, 21) is an interesting modification of both the crocodilian and the saurian arrangement. The determination of the various chambers is beset with difficulties because of the extreme variability of the separating folds.

The whole cloaca of most birds is divisible into a vestibulum, a urino-genital or middle chamber, and a rectal or innermost chamber.

The middle or urino-genital chamber is small; it receives in its dorso-lateral walls the ureters and the genital ducts, which are frequently protected by papillæ. Immediately above (headwards from) the uretro-genital orifices is a circular fold *rc*, most prominent on the ventral side; below the orifices, *i.e.*, towards the tail, is always present a well-marked fold F (*sphincter vesical* of MARTIN-ST-ANGE), best developed on the dorsal and lateral sides, whilst towards the ventral aspect it goes over into the coating of the copulatory organ, when such is present; sometimes, however, the fold is nearly circular, and very distinct. The room between this fold and the outer anal opening is homologous with the vestibulum of other Amniota; it lodges the copulatory organ; a wide opening in its dorsal wall leads into the bursa Fabricii. The entrance to this pouch is sometimes, *e.g.*, *Struthio* and *Leptoptilus*, guarded by a valvular fold, and divides the whole of the vestibulum, according to GEOFFROY, into a *bourse du prépuce* and a *bourse accessoire* (bursa Fabricii); this fold is, however, unimportant, and absent in many birds. That the bursa often forms a mere dorsal dilatation of the vestibulum has been shown and explained by FORBES. Near the sides of the penis, in various positions, are often found in both sexes glandular pores (COWPER's glands, GEOFFROY), reminding us of similar pits in the *Crocodile*. They occur, however, also in birds which possess no copulatory organ, and seem, therefore, to belong to the vestibulum itself; their analogy with anal glands of other vertebrata seems remote. The third chamber is situated above the urino-genital one, and this presents some difficulties. In most birds it forms an oval dilatation of the rectum, and is of considerable size. In *Casuarus* and *Rhea* it goes gradually over into the rest of the rectum, and its inner walls agree in structure with the latter, but in many *Carinates*, and in *Struthio*, the cephalic end of this chamber is marked by a very well developed circular fold and sphincter-like constriction, and, in connexion with this, the inner structure of the walls is smooth and very different from that of the rectum. Transitional stages are, however, numerous. Moreover, in *Struthio* this chamber is

followed by another smaller and less defined one. The succession of the chambers in *Struthio* therefore resembles much that of certain Saurians and that of very young Crocodiles.

It follows, from the arrangement described above, that in birds the urine is not retained in the small urino-genital chamber, but that, like in lizards and snakes, it passes into the next compartment above. Through this pass, of course, in all birds the fæces; if the latter are very loose and watery, like in the raptorial birds, ducks, herons, cormorants, they collect in the then very capacious room, together with the urine, and transform it into a cloaca. If the fæces are more resistant, *e.g.*, in geese, they are generally retained in the rectum, above fold *r*, and simply pass through the cloaca, unless, as in sitting birds, an unusual accumulation of excrements takes place. In the *Ostriches* defæcation and micturition are mostly separate acts, especially when through a large development of the bursa Fabricii a physiological (dorsally situated) bladder is produced.

The *Chelonia* (fig. 10, 11, 24, 25) represent a type somewhat intermediate between that of the *Ratitæ* and that of the *Monotremata*, at the same time bearing slight resemblances to that of the *Saurii*. The rectum is separated from the cloaca by a very distinct circular inner sphincter, *rc*, fig. 25. The genital ducts and the ureters open separately into a wide urino-genital sinus, which through a wide neck leads into the large ventral urinary bladder; on the other hand it stands in communication with the cloaca by a large aperture. This aperture is surrounded by a partly transverse, but chiefly longitudinal, horizontal fold, the right and left halves of which can by approaching each other completely close the urino-genital sinus, and in fact do so firmly in the living animal. The walls in the recessus recto-vesicalis project over the opening of the sinus, as shown in figs. 10, 11, 25, and 26. In the female they generally do not extend far enough towards the tail to reach the root of the clitoris, because this organ is, when very small like in *Chelys*, very far removed from the sinus. In the male, however, the crura penis extend so far back towards the rectum that the end of the dorsal groove of the organ can, with the help of the folds, receive the sperma. The fold *p*, namely, is continued (*cf.* p. 1, fig. 11) into the loose sheath-like covering of the penis, and gradually passes over from the margins of the longitudinal groove towards the dorsum penis, and near the glans it goes over into the thin ventral walls of the vestibular portion of the cloaca, as visible in fig. 10, near the insertion of the *M. retractor penis*. A result of this somewhat complicated arrangement of this cloacal-penial fold is that the copulatory organ in its proximal portion is situated rather outside the cloaca, whilst the terminal portion, or glans, is freely projecting into the cloacal lumen. Moreover, when the organ is relaxed and withdrawn, the whole cloacal-penial fold surrounds the organ like a rudimentary preputial sheath, which then bears a considerable resemblance to the conditions in *Ornithorhynchus*, fig. 27.

We can reduce the *Chelonian* cloacal type to the general *Sauropidan* arrangement

by the assumption that the ventral portion of the original chamber U D has been developed and partly shut off from the rest. Through the development of such a sinus urino-genitalis, the separation into a ventral or urino-genital-copulatory, and into a dorsal or fæcal portion of the "cloaca," is introduced, although imperfectly. At any rate there is no longer any retention of urine and of fæces in one common chamber: micturition and defæcation have become separate acts which exclude each other. Cf. also p. 11.

Paired anal pouches (cloacal bladders, anal sacs, &c.), opening by wide apertures into the dorsal wall of the cloaca opposite the urino-genital sinus, are present in the amphibiotic Emydes and in the more aquatic Chelydæ, but absent in the terrestrial Chersidæ and in the marine Cheloniidæ, which latter have their feet transformed into fins. In most Mud Tortoises (Trionychidæ) they seem likewise to be absent. HOFFMANN, however, found them in a male *T. ægyptiacus*, but not in a female *T. sinensis*.

These pouches are so placed that they can be compressed by the abdominal muscles and by the retraction of the hinder extremities, indirectly also through a peculiar mechanism in connexion with the M. lumbo-caudalis. Their walls possess often a considerable layer of circular and longitudinal non-striped muscles; their inside is sometimes villous, mostly smooth, but never glandular. The orifices of these pouches can be brought into direct communication with the cloaca, to the complete exclusion of all other openings, except the external anus (ANDERSON).

ANDERSON, and BRIDGE follows him, considers them as the "structural equivalents" of the anal musk-glands of the Crocodilia, but he adds that he "never particularly observed that the Chelonia possessing these pouches are more characterised by a peculiar odour than the pouchless forms." They frequently yield a yellowish grumous substance, most especially abundant in those forms which have these bladders provided with villi (Platysternum). This comparison is erroneous, as already DUVERNOY has pointed out; the pouches are certainly not glands, and are developed from the middle portion of the cloaca, whilst the organs of the Crocodiles are skin glands, like those on the throat.

It has been known, since TOWNSON, that some Chelonia draw water into the cloaca per anum. He put an Emys into coloured water, and observed that, when put afterwards into clear water, it vented coloured water. He concluded from this that water was pumped into the anal pouches, and that the latter served for hydrostatic purposes. This view has been generally accepted, and is strengthened by the fact that neither the true terrestrial Tortoises nor the marine Turtles with their specialised flippers possess such additional hydrostatic organs.

It was, however, apparently never ascertained if the pouches, and not only the cloaca, were filled with water. ANDERSON, with his great experience of Tortoises, remarks expressly that, although he had examined, immediately after death, nearly a hundred individuals of South Asiatic Emydes, yet in no instance had the cloacal

bladders been distended with water, whereas they often yielded the grumous substance mentioned above.

To ascertain this, I put a muzzled *Emys europæa* into a large pan of water coloured with indigo-carmin in suspended form. When taken out the following day, coloured water mixed with small blue clots was freely squirted out, followed by clear urine after pressure upon the xiphiplastron. This was repeated on several days. After four days I took the Tortoise out and at once clamped the anal opening. P. M. dissection showed that no coloured fluid had entered the intestines through the mouth. No blue stuff had entered the urinary bladder, which was half full, nor had it passed into the rectum or into the oviducts. The vestibulum cloacæ and the pouches contained a little coloured fluid, and each pouch was to the greater part filled with a large piece of clotted indigo-carmin. This could not have been collected there unless the Tortoise had frequently taken in water.

Peritoneal canals exist in all Chelonia. Their abdominal openings are situated in a recess of the peritoneum close to the sides of the neck of the bladder. ANDERSON has done much to clear up the great diversities contained in the descriptions of these organs by various anatomists; discrepancies which are less due to faulty observation than to too hasty generalisations. CUVIER described the peritoneal canals as terminating blindly near the glans penis in the male. ISIDORE GEOFFROY ST. HILAIRE and MARTIN believed that, as ANDERSON puts it, the canals divide at their extremity into two branches, one going into the cloaca, and the other tending towards the corpus cavernosum, in this way, that it opened into the cavity of the corpus cavernosum in Tortoises, whilst it terminated in a cul-de-sac in the Crocodiles. OWEN adheres more or less to this view. RATHKE does not mention these canals. STANNIUS says that in the Chelonians these peritoneal canals "are apparently, without exception, closed at their ends."

ANDERSON, after most carefully conducted experiments, expresses himself cautiously: "I am not prepared to go the length of saying that there is invariably a communication between the peritoneal canals and the cloaca in the males; but at the same time there can be no doubt that in the males of *Geomyda grandis*, *Emys Hamiltoni*, and *Trionyx ocellatus* such a communication does exist. In this respect these animals conform to the course of these canals in the Crocodile. . . . All I insist on is that in the males, as in the females, experimented upon, these canals do open into the cloaca, and in this respect conform to the general type of structure distinctive of the peritoneal canals of Crocodilia, and of the so-called abdominal pores of the Cyclostomata and Ganoid Fishes."

HOFFMANN, in BRONN'S 'Thierreich,' the latest writer on Chelonia, leaves the whole question open, but adds that he found, like CUVIER, STANNIUS, OWEN, MAYER, and LATASTE, that the canals of *Emys*, *Testudo*, *Chelys*, *Chelodina*, terminate blindly, and open neither into the cloaca nor through the glans penis.

My own investigations show the following results:—In a large male *Testudo*

microphyes (one of the Elephantine Tortoises) each canal was continued as a round tube inside and along the walls of the groove of the penis and ended blindly in its glans; when injected, or blown up from the peritoneal end, they admitted easily my fifth finger. Side branches or openings towards the outside, through the penis, did not exist, nor was it possible to force any fluid or air into the corpora cavernosa, although the specimen was fresh. This agrees with FRITSCH's account of a male *Testudo elephantopus*, and with MAYER's males of *Testudo græca* and of *Chelone midas*.

In a large and likewise fresh female *Chelys matamora* the canals extended along and in the ventral cloacal wall to open near the glans of the very rudimentary clitoris; they admitted a crow's quill throughout their length. The preparation is now in the Cambridge Museum.

A female specimen of *Testudo græca* (Cambridge collection) likewise shows a bristle passed through the canal. In the males I found the abdominal opening very narrow, whilst the canals in the penis are wide and terminate blindly in the glans. This agrees with MAYER's male specimens, and with LATASKE's male *Testudo nemoralis*, who, however, came to the conclusion that the canals never opened externally. Regarding the females, my observations completely agree with ANDERSON's experiments made on females of *Trionyx*, *Chitra*, *Batagur*, *Emys*.

To sum up, the peritoneal canals in female Chelonians open into the vestibulum cloacæ on or near the base of the clitoris. In the males they extend, without having communication with the cavities of the corpora cavernosa, and without ramifications, through the penis, and either terminate blindly in the glans (*Testudo elephantopus*, *T. microphyes*, *T. græca*, *T. nemoralis*, *Chelone midas*), or they open into the cloaca through a small orifice "situated at the base of the glans and close to the inside of the genito-urinal groove." ANDERSON (*Gecemyda*, *Emys*, *Trionyx*).*

Probably all the Chelonia possessed open peritoneal canals. Considering the small size and the position of the clitoris, it is not astonishing that the canals remain more superficial and retain their openings, whilst they are completely surrounded by the much larger male organ, and that their terminal orifices sometimes become closed.

It is, we trust, not too hasty a generalisation to say now that the canals are closed in the males of the exclusively terrestrial and marine species (*Chersidæ* and *Cheloniidæ*), but that they are open in all females and in the amphibiotic and fresh-water Chelonians.

The function of these canals has always been a puzzle. That they have a function is more than probable. I observed in a female *Chelys* a condition similar to that mentioned by ANDERSON in other species: "The peritoneal canals have their inner walls more or less coloured near their distal ends with fine dark lines of the same

* HOFFMANN says that according to ANDERSON the canals open outwardly also in the male of *Testudo platynotus*, which of course would be a rather serious exception to the other Testudines, but ANDERSON never mentions a male *Testudo*.

pigment as that of the clitoris itself, thus indicating the continuity of the living membrane with that of the external surface. The canal was also partially filled near its end with a grumous substance, but quite different from the coagulated blood that filled the corpus cavernosum."

DUMÉRIL and BIBRON suggested that the animals pumped water through these canals into the abdominal cavity, to counteract the effects of too much evaporation during the hot season. To this view ANDERSON assents. But this hypothesis must fall, first, because in the very species, which possibly might need such an arrangement, viz., the Land Tortoises, the male canals are closed; and, secondly, because the often extremely narrow and frequently papillary external orifice suggests that nothing can pass inwards, whilst the reverse is practicable. Moreover, to receive swamp water into the abdominal cavity would imply the greatest danger.

GEOFFROY ST. HILAIRE and MARTIN gave a better explanation, viz., that any fluid, which somehow or other might collect inside the peritoneal cavity, could be drained off, and that consequently Tortoises could not suffer from dropsy unless the canals were obliterated, but this suggestion was marred by the addition that "le liquide, probablement séreux, que transmettent ces canaux, doit être porté en grande partie dans les corps caverneux, d'où il semble qu'il puisse refluer dans les veines." To this mistake they were necessarily led, because they thought there existed communication of the canals with the cavities of the corpora cavernosa.

MAYER found, I think, the right solution for those males in which the canals are closed at their extremities; the serous fluid can be pressed into the penial canals and thus assist erection. In Tortoises there is often a considerable quantity of serous fluid in the peritoneal cavity. I extracted from a perfectly healthy male *Testudo græca*, immediately after death, 10 cubic cm. of a fluid, which analysis showed to be serous, whilst perhaps the same amount of fluid remained in the animal. 20 cubic cm. is certainly a great quantity for an animal not larger than the common Land Tortoise. However, when the canals are open, like in the female, their function must, like in the Crocodiles, be a different one. In this case they can only serve as a sort of safety outlet for the fluid when the abdomen is overfilled with eggs, or if, for some unknown reason, too much of this precious fluid has been accumulated; the latter alternative applies to the male, unless we allow for the persistence of the canals by inheritance from the other sex. This suggestion, although made with great reluctance, is hitherto, nevertheless, the only one that can stand anatomical and physiological reasoning.

If we homologise the peritoneal canals with the abdominal pores of Fishes, they were first used as outlets for the sexual products, i.e., they were in the service of the generative system. In the same service, although modified, they are in certain male Tortoises. In the other cases they possibly drain the body cavity, and would then, if they really are remnants of segmental tubes, have returned to their most primitive function.

The Chelonian cloacal arrangement, as described on p. 21, occurs again, with slight

modifications towards a higher type, in the *Monotremata* (cf. fig. 27). The differences lie chiefly in the relative size of the chambers UD and PD, and in the circumstance that the penis is lodged in a special pouch, which communicates with the rest of the vestibulum through a narrow opening close to the ventral brim of the anus. We can derive the wall PF, which separates the penis pouch from the rest of the whole vestibulum or proctodæum, from the ventral half of the fold F of the Sauropida; the only difference is that this fold, which in Crocodiles, Birds (cf. figs. 14, 15, and 10), and Chelonians, gradually passes over into the loose coating of the penis, and thus forms the beginning of a preputial sheath, has in the *Monotremata* become considerably elongated in a longitudinal instead of a transverse direction. This I have tried to explain diagrammatically by figs. 17 to 30. Such a reduplication of the loose penial coating would almost completely separate the copulatory organ from the urodæum, and, in fact, these folds leave in the *Monotremata* a small opening near the root of the penis groove for the reception of the sperma from the sinus urogenitalis, but not for the urine and for the eggs. The walls of the urodæum, into which the urogenital sinus opens, are very thin, and the muscular, chiefly longitudinal, coating is likewise weak, with the exception of the voluntary striped muscle on its ventral aspect (fig. 28). This chamber UD, the urodæum, receives in the middle of its ventral wall the urine and the eggs, but not the sperma. Towards the vestibulum PD it is closed by a circular constriction and partly developed fold F, and the terminus of the rectum is marked by a strong circular muscle, which forms a powerful innermost sphincter *rc*.

Whilst in *Echidna* the rectum shows a large dilatation above the fold *rc*, and forms there a true rectal chamber or coprodæum for the retention of the fæces, the latter in *Ornithorhynchus* probably mix with the urine in the chamber UD, which in this genus is very capacious in opposition to the only slightly dilated rectum.

BRIDGE's remark that the existence of a complete partition between the rectum and the urino-genital opening is a characteristic point of all Mammalia, including those which possess a cloaca, is not correct, because the urino-genital sinus and the rectum are separated from each other just as much or as little in the *Monotremata* as in the Chelonia. Not much progress towards a higher type show the *Marsupialia* (fig. 28), and even some Rodents, Insectivores, and Lemurs. The chamber UD becomes considerably shortened, and at the same time the walls in the corner between the rectum and the sinus urogenitalis, represented in the Tortoise by the fold *p*, fig. 25, transform themselves into a growth, which, progressing more and more towards the anus, results in the almost complete division of the former cloaca into a dorsal or fæcal and into a ventral or exclusively urogenital chamber. The beginning of a perinæum is consequently derived from the fold *p*, i.e., a fold inwards, above, or headwards from the opening of the urogenital sinus, and is not to be confounded with the partition PF of the *Monotremata* in fig. 27; although, of course, we have to bear in mind that the fold *p* in the Tortoises, as shown in fig. 11 at *p'*, goes over into the lateral loose coating of

the penis, which, moreover, as indicated in fig. 10, is continuous with the ventrolateral wall of the proctodæum. The preputial room of the Marsupials is therefore, strictly speaking, not completely homologous with the similar room of the Monotremes. Vulva and penis of the Marsupials, and of the Placentalia mentioned above, are still surrounded by the same external fold of skin and by the same sphincter of the anus. The shallow vestibulum is the last remnant of a cloaca.

In most *Placentalia* the cloaca is abolished by the development of a true perinæum which reaches the outer surface and secures a complete separation of the anal and urino-genital openings. The vestibulum is broken up; its dorsal portion forms the anus, whilst its ventral half, owing to the close approach of the urino-genital sinus to the surface, becomes the shallow "vestibulum" in the female, and in the male it is partly recognisable as the preputial room. The labia minora with the frenulum clitoridis are a remnant and modification of the sauropidan fold F. Lastly, in the females of these Placentalia, in which, like in *Myogale*, *Talpa*, *Galeopithecus*, and certain Lemurs, the clitoris is perforated by the urethra, the sinus urino-genitalis itself is divided into a dorsal or genital and a ventral or urethral half, both openings, however, retaining their intravestibular position.

A summary of the anatomical and physiological differences presented by the cloacal region of the various Amniota is given in the following table :—

Usually the cloaca is defined as a chamber at the terminal portion of the rectum, into which open the rectum, the ureters, and the genital tubes.

BALFOUR remarks that "in all Vertebrata the cloacal section of the alimentary tract, which receives the urino-genital ducts, is placed in communication with the exterior by means of an epiblastic invagination constituting the *proctodæum* (vestibulum cloacæ, s. anal chamber). The original boundary between the epiblast of the proctodæum and the hypoblast of the primitive cloaca becomes obliterated after the two have become placed in free communication. The hypoblastic section of the cloaca of birds, which receives the openings of the urino-genital ducts (our chamber U.D.), is permanently marked off by a fold from the epiblastic section or true proctodæum, with which the bursa Fabricii communicates." This fold is the one described in this essay as F. It occurs with modifications in all Sauropida, and even in the Mammalia. The fold *rc* separates the primitive cloaca from the rectum. Considering, first, that in the Crocodilia the genital tubes open decidedly into the proctodæal portion; secondly, the configuration of the Chelonian cloaca; thirdly, the occasional use as urinary receptacle of the bursa Fabricii; lastly, the condition prevailing in the Monotremes, we have to conclude that the vestibulum forms part of the cloaca.

The whole cloaca consequently consists of three successive chambers, which may be distinguished as follows :—

I. *Proctodæum* (P.D.), epiblastic = Vestibulum cloacæ, anal chamber, chambre copulatrice, bourse du prépuce, bourse de copulation, vestibule génito-excrémentiel.

With its derivatives : 1. Bursa Fabricii.
 2. Various hedonic glands.
 3. The copulatory organ or organs, the at least partly epiblastic nature of which is indicated by the frequently developed horny armature of the glans.

II. *Urodæum** (U.D.), hypoblastic = Primitive cloaca, middle or urino-genital chamber, vessie urinaire, canal uréto-sexuel.

With its derivatives : 1. Urinary bladder, ventral.
 2. Cloacal s. anal sacs of Tortoises, dorsal.

III. *Coprodæum** (C.D.), hypoblastic = Rectal or innermost cloacal chamber, poche vestibulaire du rectum, vestibule rectal.

The Urodæum is the oldest portion of the whole cloaca, then follows the Proctodæum, and lastly the Coprodæum has secondarily assumed cloacal functions.

* I propose to designate the typical urino-genital and the faecal chambers the Urodæum and Coprodæum in accordance with Professor E. RAY LANKESTER's terms Stomodæum and Proctodæum ; cf. 'Quart. Journ. Micr. Sci.,' April, 1876.

MODIFICATIONS OF THE CLOACA OF THE AMNIOTA.

| | Coprodeum contains— | Urodæum receives— | Proctodæum. | |
|---|--|--|--|--|
| | | | Anal opening. | Copulatory organs. |
| Sauri, A | Fæces and urine . . | Urino-genital ducts; bladder present. | Transverso | Double, dorso-lateral. |
| " B | " | Urino-genital ducts; bladder absent. | " | " |
| Ophidia | " | Urino-genital ducts; bladder absent. | " | Absent. |
| Hatteria | Fæces | Urino-genital ducts; bladder present. | Longitudinal | Single, ventral, with dorsal groove. |
| Crocodylia, young . . | Fæces | Urinal ducts | Genital ducts open into proctod. | |
| " adult | Both chambers are united, receive and contain the urine only. No fecal cloaca. | | | |
| Aves | Fæces and urino . . | Urino-genital ducts | Roundish, rather square | 1 Single, ventral, with dorsal groove. 2. Asymmetrical. 3. Absent. |
| Chelonina | Fæces only | Incomplete division. Sinus urogenitalis with a ventral bladder And sometimes a dorsal pair of pouches. | | Unpaired, dorsal bursa Fabricii. Single, ventral, with a dorsal groove. |
| Monotremata | | Urodæum and Proctodæum. | | Single, ventral. |
| " ♀ | | Ventral. | Ventral. | |
| " ♂ | | Sinus urogenitalis with bladder Passes urine, fæces, and eggs Urine and fæces | Clitoris chamber. Penial chamber passes sperma. | |
| Echidna | Dilated pouch contains fæces. | Dilatation collecting fæces and probably urine. | | |
| Ornithorhynchus | No dilatation | | | |
| Marsupialia and certain Placentalia | Fæces only | Sinus urogenitalis with bladder. Rest of urodæum lost | Reduced to a much shortened vestibulum, with exception of a large penial chamber | Penis single, ventral, with a dorsal canal (not on the dorsum). |
| Placentalia | " | Sinus urogenitalis with bladder Rest of urodæum lost | Dorsal portion forms part of anal opening, ventral vest, preputial room | " |

IV. *On the presence of MUELLERIAN Ducts in the Males and of WOLFFIAN Ducts in the Females of young Crocodilia.* (Figs. 6 and 7.)

RATHKE, in his 'Untersuchungen über die Entwicklung und den Körperbau der Krokodile,' p. 191, said that he was unable, from the embryo of *Alligator sclerops*, to ascertain if the canal running along the WOLFFIAN body was a MUELLERIAN duct or not. He was doubtful about the sex of his embryo.

In a male specimen of *Alligator mississippiensis*, of a total length of 25 centims., I found that the canal, which crosses the vas deferens ventrally and runs along the lateral side of the testis, was slightly rounded off at its upper end and seemed to be still open, although no bristle could be made to pass through it. This whole MUELLERIAN duct was considerably raised above the level of the WOLFFIAN body and duct, and was attached all along its length to a very thin and pigmentless peritoneal fold.

In another male specimen, of a total length of 33 centims., only the smallest trace of a remnant of the MUELLERIAN duct could be made out on the upper and lower ends, whilst the middle portion had already become obliterated. This remnant of the duct was no longer prominent, but was imbedded in the same peritoneal fold as the vas deferens.

Female Alligator, total length 29 centims.—The ovary was still quite smooth on its surface; along its lateral edge ran, closely imbedded in it, a light-coloured string, which could be distinctly followed between the ureter and the oviduct into the cloacal wall. Its upper or cephalic end projected a little beyond the anterior end of the parovarium, and was attached to a peritoneal fold like in the males. This is undoubtedly the remnant of the WOLFFIAN duct. Laterally from the latter, fastened on to a loose peritoneal fold, ran the oviduct and opened into the cloaca a little ventrally from the WOLFFIAN duct.

In the *Chelonia* the persistence of these ducts has been described and figured by VAN WIJHE. Fig. 10 shows the remnant of the MUELLERIAN ducts in a full-grown male specimen of *Testudo graeca*. In the adult male *Hatteria* no trace could be found.

General Conclusions.

The want of an intromittent copulatory organ is in the various Vertebrata met in very different ways. The fundamental difference between the Selachian pterygopodia and any other type of copulatory organs is plainly indicated by the nerve supply, and wherever in the Amniota skeletal muscles enter into the service of copulation they have become, as shown above (cf. pp. 10–14), secondarily attached to the true penes long after the latter had been developed from the walls of the alimentary canal.

The first indication of an intromittent organ in the *Amphibia*, according to WIEDERSHEIM, is a small unpaired papilla on the dorsal wall of the anal or vestibular

portion of the cloaca. It is found in Urodela only, *e.g.* in *Salamandrina perspicillata* and in *Triton platycephalus*. In the latter it consists of an erectile cone, which WIEDERSHEIM considers as derived from the caudal portion of the cloacal lips. This dorsal unpaired, median, still very rudimentary organ, is a type restricted to Amphibia.

Another type of low standing is met with in the Gymnophiona and to a less pronounced degree in Hatteria. The immissio seminis is secured by the finger-of-a-glove-like eversion of the cloacal walls, chiefly of those of the proctodæum. This type is best developed in the Gymnophiona. In them a great portion of the cloaca can through its strong muscular coating be converted into a tube projecting several centimetres, and is then used as an intromittent organ. A bifurcated M. retractor, which arises from the abdominal walls, is attached to the recessus of the middle portion of the cloaca. In the female the cloaca is short and cannot be protruded (*cf.* WIEDERSHEIM, 'Anatomie der Gymnophionen,' figs. 88 and 89, Taf. ix.).

From this stage, which probably represents that of the Proreptilia and of the true Reptiles before their separation into Crocodilia and Chelonia and into Sauria and Ophidia had taken place, we can, I think, derive the types of the other Amniota.

The definition that the copulatory organs of the Lizards and Snakes are connected with the posterior or dorsal, but those of the other Amniota with the anterior or ventral wall of the cloaca, seems to have led to the misconception that no homology can be traced between these two types of penes.

To clear this question up, I draw attention to the following considerations:—

1. In Hatteria and in the Gymnophiona, as explained above, the inner walls of the cloaca form by protrusion and evagination a temporary intromittent organ.

2. In the embryos of Lizards the anus is still round, and not a transverse slit. On each side, the lateral inner walls of the proctodæum are, together with part of the uro-anal fold F, raised up into prominent cones, which afterwards are invaginated and withdrawn into the post-anal region of the tail by the action of specialised muscles. In connexion with this the anal opening assumes its typical transverse shape.

In the Snakes, which are the most specialised branch of the Saurian stock, the original position of the penes is cœnogenetically obscured, although still traceable.

3. The original duplicity of the "unpaired" copulatory organ of the other Amniota is still indicated by the nerve supply, the vascular supply, by the corpora cavernosa s. crura penis, by the double penis and clitoris of certain Marsupials, like Didelphys, and by the fact that in the Chelonia the peritoneal canals are continued far into the lateral portions of the penis.

4. The completely divided right and left penes of the Lizards and Snakes are supplied by the same nerves as are the copulatory organs in all the other Sauropida.

These circumstances, considering also the modifications of the cloaca as described in this paper, seem to me to permit the following conclusions:—

That in all Amniota the intromittent organs, no matter if double or single, are derived from the walls of the outermost cloacal chamber, in connexion with the

separating uro-proctodæal fold F. The epiblastic origin of the proctodæum explains the frequent presence on the copulatory organs of epiblastic products, like horny spines, scales, sebaceous glands, and its highly sensitive nature.

That there existed a stage of phylogenetic development, during which the lateral portions of the protrusible tube became stronger, and that they specialised themselves into a right and into a left imperfect intromittent organ, the walls of which then—being stowed away in recessus of the proctodæum—escaped being used also as the walls of the efferent fæcal chamber.

That subsequently in one group, viz., in Snakes and Lizards, these penes were shifted back towards the tail and were developed into separate organs. After this had taken place they could not well meet and fuse with each other in the posterior dorsal corner of the anus, since then their bases would be too far removed from the openings of the vasa deferentia, with which they are now still connected by longitudinal folds forming the continuation of their semi-canals.

That in the other groups of Amniota the two primitive lateral erectile flaps approached each other towards the ventral side, and thus arrived at the anterior or ventral side of the cloaca. Their fusion, beginning probably at the basal part, which at the same time was further withdrawn from the surface, secured the reception of the sperma from both vasa deferentia into one canal.

That this ventral copulatory organ, now in the Reptilia restricted to the Crocodilia and Chelonia, has been inherited by the Avian stock, and has been specialised in the various ways fully and correctly described by JOH. MUELLER. The struthious form comes nearest to that of the monimostylic Reptiles, whilst that of the other Ratitæ and of the Lamellirostres shows great specialisation in being evertible. The comparison of the organ, as it is found in various other Carinatae, e.g., in the Tinamoes, the Cracidæ, in Phoenicopteris, Platalea, Ciconia, shows a gradual diminution in size and a simpler structure, with all the appearance of a degraded organ. Lastly, in the majority of Birds, especially in the highest, it has disappeared, and the primitive way of everting the cloaca is again resorted to. The degeneration and final loss of such an organ, the development of which must have been caused and favoured equally by natural and by sexual selection, is a fact which we would not have arrived at by *a priori* considerations.

That the presence of a clitoris is not due to an originally hermaphroditic condition, but to direct paternal inheritance, and that it is preserved because of its hedonic nature.

That the extraordinary resemblance of the copulatory organs and the various cloacal chambers of the Monotremata to those of the Chelonia and young Crocodilia can hardly be explained by homoplastic coincidence, but that it strongly urges the phylogenetic relationship of the Mammals with the Reptiles. This, however, is only one more link in the long chain which, being anchored in the triassic Theriomorpha, in spite of Mono- and Amphicondylism, makes the Amniota more akin to each other than to the Amphibia.

EXPLANATION OF PLATES.

PLATES 2-5.

Fig. 1. *Crocodylus vulgaris* (nat. size).—Ventrilateral view of the penis; the greater portion of the outermost cloacal chamber has been cut away.

Gl. = left musk gland.

Fib. = fibrous band connecting the penis with the symphysis ischium.

Per. = continuation of the body cavity as peritoneal canal; a probe has been passed through the left canal.

This specimen is in the Royal College of Surgeons, London.

Fig. 2. *Crocodylus* sp. ? (nat. size).—Ventral view of the cloaca and of the female genital organs; the cloacal ventral wall has been partly cut away and the anus has been opened out, the clitoris now lying on one side. The peritoneal folds and the right oviduct are removed.

This specimen is in the Museum of Comparative Anatomy, Cambridge (No. 1377A).

Fig. 3. *Alligator mississippiensis*, ♂ (nat. size).—Left lateral view of the rectum, the cloacal muscles, and of the plexus pudendus.

B. P. = bulbus penis.

Sph. = m. sphincter.

trans. m. = m. transversus medianus.

tr. per. = m. transversus superficialis s. m. perinei.

pr. cl. = the unstriped muscle, described on p. 14.

v. c. = vena caudalis.

a. c. = arteria caudalis.

Sy. lat. = chain of the N. sympathicus lateralis.

S = N. sacralis = N. spinalis xxvi.

a = first presacral nerve.

α = first postsacral nerve.

cd. fm. = nerves to the m. caudi-femoralis.

v. d. = vas deferens.

u = ureter.

Fig. 4. *Alligator mississippiensis*.—The nerve branches sent from stems α and β to the penis, the anal chamber, to the lateral (R. l.) and to the dorsal wall (R. d.) of the rectum; slightly enlarged.

Fig. 5. *Alligator mississippiensis*, ♂.—The cloacal muscles seen from the right ventral side after removal of the m. rectus lateralis.

Sy. isch. = symphysis ischium.

transv. sup. = m. transversus superficialis.

transv. med. = m. transversus medianus.

sph. = m. sphincter ani.

α = the nerve branch from stem α which supplies the m. rectus lateralis, the mm. transversi, and the m. sphincter.

pr. cl. = the unstriped protractor muscle.

cd. isch. = m. caudi-ischiadicus.

Fig. 6. *Alligator mississippiensis*, young ♂.—Ventral view, left side, twice nat. size, showing the left ureter, vas deferens, and the Muellierian duct.

Fig. 7. *Alligator mississippiensis*, young ♀, slightly over nat. size, showing the oviduct and the remnant of the Wolffian duct.

Fig. 8. *Crocodylus biporcatus* (nat. size).—The left half of the pelvis, together with the limb and the left half of the tail, are removed.

r = position of the fold r , forming the inner margin of the coprodæum.

Fig. 9. *Crocodylus acutus* (nat. size).—Ventral view after removal of the skin.

Fig. 10. *Testudo græca*, ♂ (nat. size).—The penis is stretched out, and, after removal of the pelvis, although still adhering to the cloacal walls, has been pushed over to the left side.

pb. = symphysis pubis.

pc. = visceral opening of the peritoneal canal, the course of which in the penis is dotted in.

R = rectum.

Bl. = the cut-off walls of the neck of the urinary bladder, to show its communication with the cloaca and with the longitudinal furrow of the penis. On the pigmented epididymis is seen the remnant of the Muellierian duct.

retr. p. = m. retractor penis.

pb. cd. = m. pubi-caudalis.

Fig. 11. *Testudo græca*.—Penis stretched out and laid on to the plastron to show the groove, the orifice of the uro-genital sinus, and the opening of the rectum, which is surrounded by the fold rc .

Fig. 12. *Hatteria punctata*, ♂ (nat. size).—The cloaca has been opened slightly to the left from the medio-dorsal line, and its walls are laid asunder. The left kidney and genital apparatus only are figured.

Bl. = urinary bladder, which was still connected with the end of the left lobe of the liver by a ligamentous stalk, the obliterated allantoic vein.

rc = fold separating the coprodæum from the urodæum ; the hole in the middle is the opening of the bladder.

F = fold separating the urodæum from the proctodæum ; on this fold are seen the two papillary flaps, into which are projecting the remnants of the peritoneal canals.

This preparation is in the Cambridge Museum.

Fig. 13. *Hatteria punctata* ♀ (nat. size).—Younger specimen ; ventral view. bladder and neck of bladder cut off.

F = the fold between urodæum and coprodæum, with the median dorsal flap, which fits into the opening of the bladder.

u. g. = the united left openings of the ureter and oviduct below the base of a papilla.

Gl. = the left anal gland.

This preparation is in the Cambridge Museum.

Fig. 14. *Rhea Darwini*, ♂ (reduced size).—Ventral view into the urodæum ; a window has been cut into the ventral wall of the rectum.

rc = fold shutting off the rectum from the urodæum ; in the latter are seen the papillæ with the uretral openings ; the walls of this chamber are continued into the crura penis.

B. F. = bursa Fabricii.

This preparation is in the Cambridge Museum.

Fig. 15. *Rhea Darwini*, ♂ (the same specimen as Fig. 14, right side-view).—The right half of the rectal wall, of the urodæum, and of the proctodæum has been cut away to show the relation of the various chambers to each other and to the half-protruded penis.

sph. = m. sphincter ani.

lev. an. = m. levator ani.

R = ventral wall of rectum.

V = ventral corner of anus.

B. F. = bursa Fabricii.

Fig. 16. *Leptoptilus argala*, ♂ (nat. size, ventral view).—The greater part of the ventral walls of the cloaca has been cut away. For this drawing I am obliged to W. F. R. WELDON, M.A.

Figs. 17–29. Diagrammatic representation of the chief modifications of the cloaca, seen from the right side.—The dorso-median line looks towards the left, the ventral median line to the right side in the drawings.

CD = coprodæum = *poche vestibulaire du rectum* or *ressie urinaire* (Struthio), GEOFFROY ST. HILAIRE ; = *vestibule rectal*.

CD' = additional rectal faecal chamber = *vestibule rectal* of GEOFFROY in Struthio.

UD = urodæum, coloured light-blue throughout the series = *ressie*

urinaire, GEOFFROY; or, *canal urétro-sexuel* in Struthio; *eigentliche Kloake*, BUDGE; *loge uro-génital*, RETTERER.

PD = proctodæum = *bourse de copulation* or *bourse du prépuce*, GEOFFROY; *Vorhof der Kloake*, BUDGE; *vestibule génito-excrementitiel*, DUVERNOY; *poche postanal + passage anal*, RETTERER.

F = the fold between the urodæum and the proctodæum, shaded grey = *le véritable col de la vessie*, GEOFFROY; = *repli uro-anal*, RETTERER.

rc = the fold between the urodæum and the coprodæum, coloured dark-blue = *premier bourrelet ou le véritable anus*, GEOFFROY; = *repli uro-rectal*, RETTERER; *sphincter rectal*, MARTIN-ST.-ANGE.

rc₁ = the fold which shuts the inner end of the coprodæum, shaded with vertical lines.

sph. = m. sphincter ani, shaded with crossed lines.

p = the copulatory organ, shaded grey.

Ur. = ureter.

Gen. or v. d. = genital duct.

B. F. = bursa Fabricii = *bourse de Fabrice* or *bourse accessoire*, GEOFFROY.

AS = anal pouch or anal sac.

Fig. 17. *Lacerta ocellata*, ♂.—Type A, with a urinary bladder. In figs. 17–19 one of the paired copulatory organs only is shown.

Fig. 18. *Monitor*, ♂.—Type B, without a urinary bladder.

Fig. 19. Typical snake, ♀.—*Tropidonotus*.

Fig. 20. Typical bird.

Fig. 21. *Struthio*.

Fig. 22. Crocodile, adult.—Anal glands not figured.

Fig. 23. Crocodile, very young, or embryonic.

Fig. 24. *Emys*.—Penis stretched out.

Fig. 25. *Testudo*.—Penis withdrawn.

Fig. 26. *Ornithorhynchus*, ♂, adult.

Fig. 27. *Ornithorhynchus*, ♂.—Transverse section through the middle of the urodæum.

Fig. 28. *Macropus giganteus*, ♂, adult.—Retr. = m. retractor penis.

Fig. 29. *Anthropoids*, ♀.

Fig. 30. *a–f*.—Diagrammatic representation of six stages of the phylogenetic development of the sinus uro-genitalis, and the gradual formation of a ventral chamber or uro-gonodæum.

The black line indicates the course of the sperma.

The blue line indicates the course of the urine.

a. Crocodilian stage.

- b.* Hypothetical stage, intermediate between Crocodiles and Tortoises. An analogous, but dorsal, recessus uro-genitalis is developed in Lizards and Snakes.
- c.* Chelonian stage.
- d.* Hypothetical stage, intermediate between *c* and *e*.
- e.* Monotreme stage.
- f.* Marsupial stage.

III. *On the Changes in the Proteids in the Seed which accompany Germination.*

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A CURIOUS and characteristic feature of the life-history of the higher forms of plants is the long resting period which takes place in the seed, following the reproductive act, after a certain amount of development. Fertilisation of the female element is succeeded by a period of activity, during which great changes in the oosphere take place, many new cells are formed, and the new individual becomes recognisable. But then comes a remarkable alteration; the development for a time is arrested, no new cells are produced, but those already formed, which constitute chiefly the first leaves or cotyledons of the new plant, become filled with nutrient materials, forming reserves upon which, after the resting period, the young plant will subsist, and which will enable it to resume its growth. Or it may be that the nutrient material may be accumulated in cells immediately surrounding the young embryo, cells which form the so-called endosperm and which are not actually part of it. A curious feature this, not represented exactly by anything in the cycle of animal life, though perhaps the condition of the egg which is deposited by the parent and quickened later into active life approaches somewhat to it. This differs greatly, however, in the length of the quiescent period, which in the seed may be almost indefinitely prolonged. What changes, if any, take place in the cells during this period is not known and cannot well be ascertained. At the end of this time changes do take place, and the arrested development is resumed. That the condition of things inside the seed is not exactly alike always, seems pointed to by the fact that seeds of the same plant do not germinate at all times with equal readiness, though exposed to the same favourable conditions. During this period, long or short as it may be, and its length varies extremely, the different bodies occupying the interior of the cells of the cotyledons or the endosperm maintain their character apparently unchanged, or, if changed at all, the nature of the change is such as not to be recognisable by microscopic examination or by chemical analysis, only being marked by greater or less resistance to the setting up of the manifest changes which are known as the process of germination.

The process of deposition of the several reserve products in the cells of the seed has been watched by many observers, and the details of the storage have been examined

and traced out step by step. The resumption of the arrested development, under the conditions of moisture and of temperature which we call favourable to germination, involves intricate metabolic processes in which the different materials that have been stored are all separately concerned, each group of bodies being transformed into nearly related ones which are adapted for the new conditions of life. Instead of the resting forms of proteids, carbohydrates, &c., which are not diffusible and hence cannot pass from cell to cell and so traverse the plant, we have new forms which can readily travel to those points where growth is proceeding and new cells are being formed, and hence plastic material is required. The details of these transformations are in many cases still obscure, though some facts have been ascertained which throw light upon the nature of some of the chemical processes. In the case of the starch, which is so constant a constituent in seeds, it has been proved that the formation of sugar takes place from it by the agency of a ferment, exactly as it does in the corresponding process in the animal economy. From almost any seed a so-called diastatic ferment can be obtained, and so constant is the occurrence of the latter body in vegetable organisms that it can be prepared from almost any part of plants. From analogy it would seem probable that the proteolytic changes noticeable would have a similar cause, and that from seeds in which large quantities of reserve proteids are stored evidence of such a body could be obtained.

Other seeds are noticeable in connection with the large store of cellulose which they contain in their endosperm cell-walls, a store which disappears as the process of germination proceeds, and which is no doubt made use of for the nourishment of the young plant. It seems *possible* at least that a similar ferment action may be the cause of the transformation here, particularly when it is remembered that in the intestines of many animals, notably in the herbivora, a digestion of cellulose somewhere takes place, and that probably under the influence and by the activity of bacteria, which are themselves vegetable organisms, although in their case it is hardly likely that an *isolable* ferment is prepared by them for the work. The proved existence, therefore, of diastatic ferments almost universally in plants, and the probability of existence of the others alluded to, besides the discovery of other ferments in the vegetable kingdom, not immediately connected with the process of germination, have directed investigation into the metabolic changes in the seed with the view of discovering such ferments there. In 1874 some observations were published by V. GORUP-BESANEZ, which indicated the existence of a peptone-forming ferment in the seeds of the Vetch, being there in company with another which had amylolytic or diastatic properties. V. GORUP-BESANEZ speaks of this body* as having power to convert fibrin into peptone, but he did not apparently trace its normal action in germination, as he does not indicate what changes it causes in the proteids stored in the seed. In 1875 he supplemented his observations on the Vetch in another paper,† in which he states

* 'Deutsch. Chem. Gesell. Ber.,' 1874, p. 1478.

† *Ibid.*, 1875.

he has discovered a similar body in the seeds of Hemp, Flax, and Barley. V. GORUP-BESANEZ was followed in 1878 by KRAUCH,* who says that he fails to confirm his results. In a later paper by the latter writer,† he severely criticises v. GORUP's method of working, and states that the results he obtained were due to imperfect experiments. While v. GORUP-BESANEZ says that fibrin acted on by the body he prepared from the vetch seed was dissolved, and that then the solution when filtered gave a good biuret reaction, KRAUCH insists that the biuret reaction was due to something present in the ferment solution and was readily yielded by the latter alone. He says, further, that the fibrin in his own experiments appeared to get less, but that this was due to shrinkage of the flocks of it, and not to solution. KRAUCH's work, however, appears untrustworthy, for he does not explain the disappearance of the fibrin which v. GORUP alleges, nor does he show that there was no formation of peptone in the process, although the ferment solution itself might have given a biuret reaction. With such a ferment solution to begin with, other means were necessary to detect the peptone if any were formed. KRAUCH's own control experiments were somewhat scanty.

PROTEOLYTIC FERMENT.

The nature of the ferment discovered by v. GORUP-BESANEZ was not satisfactorily established in his investigations. He calls attention himself to the fact that in the young shoots of newly-germinated plants of *Vicia*, under certain conditions, large quantities of leucin and asparagin might be shown to exist. The ease with which crystalline bodies of that nature would be able to pass through such structures as cell walls points to the probability of this rather than peptone being the form in which nitrogenous matter would pass to the growing points from the reservoirs in which it had been stored. Moreover, the fact that peptone cannot be discovered in or near the growing parts, and the almost complete indiffusibility of any other form of proteid matter, lend much support to the view that crystalline products are the ultimate expression of germinative metabolism of proteids. On these grounds, therefore, there is a great probability that the proteolytic ferment in the seeds will be found to resemble the tryptic rather than the peptic ferment of the animal organism. V. GORUP-BESANEZ was not, however, able to convince himself that the ferment in the vetch seed carried the changes in the fibrin beyond the stage of peptone.

Since that time MARTIN‡ has shown that a tryptic ferment is present in the latex of *Papaya carica*.

When v. GORUP-BESANEZ was writing, but little had been ascertained as to the nature of the proteid substances stored in seeds. RITTHAUSEN's work had apparently shown the existence of bodies differing greatly from animal proteids, and forming a

* 'Beiträge zur Kenntniss der ungeformten Fermente in den Pflanzen.' Berlin, 1878.

† 'Landwirthsch. Versuchs-Stat.,' vol. 27, p. 383.

‡ 'Journ. of Physiol.,' vol. 5 (No. 4), and vol. 6 (No. 6).

series peculiar to vegetable organisms. Inasmuch as these were not shown to approach the animal proteids in any particular direction, and on account of the paucity of the reactions known to characterise them, nothing was done in the direction of ascertaining their fate during germination.

In 1877* and 1880† WEYL and ZOLLER began to place them on the same footing as animal proteids, and to show how they resembled them, establishing the existence of globulins in seeds. About the same time a series of elaborate investigations, by VINES,‡ cleared up many points of difficulty, and gave us for the first time a clear conception of the chemical nature of these reserve vegetable proteids which exist in the seed in the form of the so-called aleurone grains. According to the latter observer, these consist of members of the great groups of the albumoses and the globulins.

The nature of the aleurone grains, or stores of vegetable proteids, now being, at any rate generally, understood, it becomes possible to ascertain something about the nitrogenous metabolism of the process of germination as a whole; to see whether it is a process of ferment action, for this really can hardly be considered established, though rendered highly probable, by v. GORUP-BESANÉZ's experiments on fibrin, the aleurone being greatly different from this form of proteid; to ascertain whether, if so, the action can go as far as the formation of crystalline nitrogenous bodies; and to trace the series of changes in the proteids which take place as the germination proceeds.

During the past year I have endeavoured to deal experimentally with those questions, and for the purposes of the investigation have taken the Lupin as, for many reasons, the most suitable. The seeds are of large size, and germinate very readily; from VINES's work the approximate composition of the proteid reserve materials is very well known; and according to v. GORUP-BESANÉZ, in plants nearly related, i.e. the Vetches, a proteolytic ferment exists. In his paper already alluded to, the latter writer states that with the Lupin he only obtained a negative result; but, for the reasons mentioned, it seemed not impossible that such a ferment existed.

The products of the decomposition of fibrin by proteolytic ferments being well understood and easily recognisable, my first experiments were directed to the action of the extract of the seeds on this body. A considerable quantity, about a quarter of a peck, of the seeds of *Lupinus hirsutus* were germinated for four days in a greenhouse, and when the radicle had grown to a length of about 2–3 inches they were removed, the cotyledons separated from the other parts, and ground in a mill. V. GORUP-BESANÉZ prepared his extracts by an elaborate process of dehydration by alcohol, extracting with glycerine, &c., several times repeated.§ This method was

* 'Zeitschr. f. Physiol. Chem.,' vol. 1, 1877. 'Deutsch. Chem. Gesell. Ber.,' Jahrg. 13, 1880, p. 367.

† 'Deutsch. Chem. Gesell. Ber.,' Jahrg. 13, 1880, p. 1064.

‡ 'Journ. of Physiol.,' vol. 3 (No. 2).

§ His extract so prepared would yet contain a large amount of albumose which is soluble in glycerine

found to answer well with Vetch seeds, but did not, for some reason, extract anything from the Lupin. Instead of following it, I only made a glycerine extract of the ground germinating seeds, which I used after dialysing for some time. In consequence of this mode of extraction it was necessary to modify the ordinary method of testing its activity. This glycerine extract would not only contain any ferment present, but would have taken up such of the proteids of the seed as were soluble in water. According to VINES, in the Lupin there occur hemialbumose and a form of globulin. The latter is soluble only in salt solutions, but the former dissolves in water readily. As this hemialbumose also gives the biuret reaction, which is always relied on as the characteristic reaction of peptone, it is evident that its presence in the extract containing the ferment would render it very difficult to say that the latter formed peptone at the expense of the fibrin, unless some method could be devised which should separate the albumose from any peptone that might be formed, or a reaction discovered which peptone gives and albumose does not. Such a method of separation, neglected apparently by both V. GORUP-BESANEZ and by KRAUCH, is furnished by dialysis. According to VINES, hemialbumose does not dialyse, while peptone does so readily. It is evident, therefore, that if a mixture of the two are at any time present in the same dialyser the peptone will pass through and be found in the dialysate, while the hemialbumose will not. Before trusting to this method, I carefully tested this asserted indiffusibility, and found I could confirm VINES completely. After more than a week's exposure of a solution of hemialbumose in a parchment-paper dialyser, the liquid outside failed to give a biuret reaction, while the solution inside did so readily. Instead of using glass vessels, therefore, for my digestive experiments, I carried them all out in well tested dialysing tubes, through the walls of which would pass, as formed, the peptones and nitrogenous crystalline bodies, should such be produced, while any hemialbumose or globulin present in the extract would be retained in the vessel. In all cases careful control experiments were carried out, all the conditions being the same in both sets, except that one set contained the ferment extract and the other did not. The dialysers, too, were carefully tested as to their intactness at frequent intervals. The fluid outside was made of the same reaction as that inside.

The experiments on which I base my conclusions were carried out in nearly every case with extracts which had themselves been dialysed with care. The reason for this was that, as germination had begun in the seeds, it was probable that in the extracts there would be a small amount of leucin or asparagin. The dialysis was continued till the dialysate gave no proteid reaction, and on concentration and evaporation on a glass slide deposited no crystals. In one or two cases extracts were used without such dialysis, but when this was the case precautions were taken against error, which will be detailed in giving the results.

In selecting the medium in which to conduct the experiment on fibrin, attention and is not coagulated by exposure to alcohol, remaining under it unchanged for a considerable period. This gives the biuret reaction, and explains, therefore, KRAUCH's criticism.

was first given to the reaction of the germinating seed. This was found to be faintly acid, and consequently a fluid containing free HCl to the amount of .2 per cent. was used. Further experiments bearing on this point will be detailed later. It was, unfortunately, not possible to reproduce the conditions obtaining in the seed, where the proteid matter is in considerable excess and but little fluid is present.

Some fibrin was taken and boiled for twenty minutes in weak HCl, to destroy any possible ferment adherent to it. It became swollen up and of the usual semi-transparent appearance. A quantity was put into a dialyser with about 30 c.c. of the dialysed glycerine extract, which was mixed with an equal bulk of .4 per cent. HCl; and the dialyser was immersed in a beaker containing 100 c.c. of .2 per cent. HCl. A control was kept by having a precisely similar quantity of the glycerine extract boiled before adding the fibrin, and another by putting a similar quantity of fibrin into .2 per cent. HCl alone. All were then placed on a water bath at 37–40° C., and left to digest. After a period of time, varying in different experiments, but in every case very much more prolonged than is necessary with gastric or pancreatic extracts, the dialysate from the tube which contained the unboiled extract of the seeds was found to contain peptone, as evinced by the pink colour given on addition to it of excess of NaHO and a drop of CuSO_4 . When this had been well marked for some time, the dialysate was boiled. No turbidity resulted, nor did any opalescence or precipitate appear on neutralisation. Alcohol gave a precipitate which settled out slowly. On concentrating the dialysate, and evaporating slowly, crystals were formed which were recognisable under the microscope as those of leucin. They were carefully compared with the crystals of leucin figured by FUNKE in his 'Physiological Atlas,' and corresponded entirely with them.* When the liquid containing these was evaporated to dryness with HNO_3 , and the residue treated with caustic soda, and again evaporated, it gave the oily drop said to be characteristic of leucin (SCHERER's test).

Besides these, others were present in less quantity, which, from their form, appeared to be those of tyrosin. The liquid containing them changed to a pale pink colour when boiled with MILLON's reagent. This was confirmatory of the presence of the latter body, though it cannot be held to be by itself conclusive, as a trace of peptone present, if not enough to be precipitated by the MILLON's reagent, would give a somewhat similar reaction.

No digestion took place in either of the control tubes.

The process was continued in this case for six days. In another experiment I worked with an extract that had not been dialysed, when I proceeded rather differently. In the dialyser I put 30 c.c. of the extract and 30 c.c. of .4 per cent. HCl. In the beaker in which the dialyser was immersed I put 120 c.c. of .2 per cent. HCl. After 24 hours I poured this away and substituted 120 c.c. of fresh HCl .2 per cent. I changed this again after 24 hours more, and again after a further

* The microscopic slides of these crystals were further examined for me by Dr. SHERIDAN LEA, who kindly allows me to quote his opinion that they were those of leucin.

24 hours. This treatment removed any crystalline bodies present in the extract used, leaving in the final dialysate only such as had been formed in the latter part of the digestion. The quantity found in the last 120 c.c. was as great as in the former experiment. On watching from time to time the progress of the action, as shown by the changes in the fibrin, a correspondence was evident between the behaviour of the lupin extract and that of pancreatic juice. True, the reaction of the fluid was different, but the fibrin seemed to be eaten away from the outside just as in pancreatic digestion, and not to gradually pass into solution, as it does when acted on by pepsin. When the digestion was complete the liquid was quite turbid, and deposited, on standing, a sediment of fine particles. In the tubes in which the fibrin was only treated with HCl it maintained throughout the peculiar, almost translucent, appearance characteristic of it almost immediately it is subjected to the acid's action, and the liquid was only slightly opalescent after days of treatment.

In no case during the experiments did bacteria appear in any of the tubes.

The course of digestion as estimated by the products formed also closely resembled that brought about by trypsin. In the process caused by the latter there are three distinctive bodies or groups of bodies formed apparently successively. The first of these is precipitated on neutralising the digesting mixture, and the precipitate is soluble in either weak acids or alkalis. To it has been given the name of parapeptone. Very soon after digestion has begun, the so-called peptone is recognisable, and leucin and tyrosin, crystalline bodies, appear. Besides these, there are formed, simultaneously apparently with the parapeptone, bodies called albumoses, which possess the peculiar properties of being insoluble, some in slightly acid solutions, some in water, at ordinary temperatures, but being soluble at temperatures higher than 70° C. In the digestions by the lupin extract the liquid in the dialyser very soon gave a conspicuous neutralisation precipitate, soluble in acids or alkalis, and being evidently parapeptone. Besides this there appeared to be a considerable amount of albumoses formed, more than is usually seen with either pepsin or trypsin. These were precipitated with the parapeptone, but could be readily separated from the latter by warming the tube containing the mixed precipitates suspended in water. A good deal of the suspended matter dissolved, and, on filtering while hot, the insoluble parapeptone was removed, while the albumose remained in solution and was thrown down again as the liquid cooled. The albumose in greatest quantity here differed somewhat from the hemi-albumose of KÜHNE, or α -peptone of MEISSNER, as this body is precipitated by acids. It corresponds more closely with KÜHNE's heteroalbumose,* which is insoluble in water, being thrown down by dialysis. A certain amount of dysalbumose also was present.

According to KÜHNE, the albumoses are partly the result of the action of the acids on proteids, for they are formed by digesting fibrin with HCl .2 per cent. for a considerable time. In this case they were not so formed, but were due to the action of the extract, for a control tube with HCl only and fibrin contained a mere trace of them.

* KÜHNE and CHITTENDEN, 'Ueber Albumosen,' 'Zeitschr. Biol.,' vol. 20, 1884, p. 11.

The subsequent or coincident appearance of peptone and crystalline bodies has already been described. For the satisfactory demonstration of the formation of these, an experiment was made on a large quantity of fibrin which was subjected for a week to the action of 25 c.c. of the extract. At the conclusion of this time the method used to separate leucin by v. GORUP-BESANEZ in his investigations was followed. The liquid was boiled, filtered, and neutralised; the neutralisation precipitate removed, and acetate of lead added. This body forms a compound with leucin which is insoluble in alkaline fluids. The precipitate so formed was filtered off, and suspended in water. The leucin and lead compound was then decomposed by passing a stream of SH_2 through it, and the sulphide of lead so formed removed by filtration. The filtrate was evaporated to dryness and extracted with boiling absolute alcohol. The last reagent would take up leucin, but not any traces of proteids that had gone down with it. The alcohol extract was then evaporated to concentration, when it deposited the crystals. The essential nature of the action of the extract on fibrin having thus been established, it becomes possible to speak of the presence in it of a proteolytic ferment and to consider this a tryptic rather than a peptic one.

Several points connected with its action were then investigated.

1. *At what temperature is it most active?*

The influence of temperature on the ferments of the animal organism is one of the most remarkable features they possess. Their action is suspended at a very low degree, gradually improves up to an optimum, which is the temperature of the animal body, and beyond this point declines, till on exposure to about 70°C . they are destroyed.

It does not seem improbable that, as the proteolytic ferment of the Lupin works naturally in a body which is not at so high a temperature as that occurring in the alimentary canal, a lower degree of heat than that would suit it best. In the experiments on the point 20 c.c. of the dialysed glycerine extract were taken and diluted with 20 c.c. of HCl of .4 per cent. strength. This was then divided into two, and a measured quantity of boiled swollen fibrin was placed in each. One was kept at the temperature of the laboratory, and the other put in a water-bath at a temperature of 37°C . After two days' digestion three-quarters of the fibrin in the latter had been digested; the liquid was turbid, and peptone in abundance present. In the former, digestion was just beginning to be evident, the edges of the fibrin only being corroded away. Two days later the warm tube contained no recognisable fibrin, while the cool one showed digestion about half completed.

Boiling the fluid which contained the ferment effectually destroyed its activity.

It therefore corresponds in its behaviour to the animal ferments, working best at a moderately high temperature, such as 40°C ., but being destroyed by too great heat.

2. *What is the medium most suitable for its action?*

In the germinating seed, as already stated, the reaction was acid, the depth of tint given to very sensitive litmus paper being about the same as that caused by .2 per cent. HCl. As the tryptic ferment in the pancreas requires an alkaline medium for its activity, experiments were made to test whether this one worked best in an alkaline fluid. Six tubes were taken, of the same capacity, and into each was put a measured quantity of boiled swollen fibrin. The tubes were labelled A, B, C, D, E, F, and to the fibrin in each was added as under:—

- A. 50 c.c. .2 per cent. HCl and 5 c.c. ferment extract.
- B. 50 c.c. .4 per cent. HCl and 5 c.c. ferment extract.
- C. 50 c.c. 1 per cent. HCl and 5 c.c. ferment extract.
- D. 50 c.c. 1 per cent. Na_2CO_3 and 5 c.c. ferment extract.
- E. 50 c.c. 1.5 per cent. Na_2CO_3 and 5 c.c. ferment extract.
- F. 50 c.c. .5 per cent. Na_2CO_3 and 5 c.c. ferment extract.

Three control tubes were also prepared, containing no ferment extract.—

- G. 50 c.c. .2 per cent. HCl.
- H. 50 c.c. .4 per cent. HCl.
- I. 50 c.c. 1 per cent. Na_2CO_3 .

To each of the latter an equal amount of fibrin was put, as in the first six. After 24 hours, and again after 48 hours, they were examined, with the results given in the subjoined Table.

| | After 24 hours | After 48 hours. |
|---|--|---------------------------|
| A (.2 per cent. HCl) | Digestion most active. Most peptone formed | Fibrin all gone |
| B (.4 per cent. HCl) | Digestion begun. Less active than A | Fibrin about half gone |
| C (1 per cent. HCl) | Digestion begun. Nearly as fast as B | About as B |
| D (1 per cent. Na_2CO_3) | No change in fibrin, except that it had lost translucency. No peptone formed. A little alkali-albumin present, due to the Na_2CO_3 | No further change evident |
| E (1.5 per cent. Na_2CO_3) | As D | As D |
| F (.5 per cent. Na_2CO_3) | As D | As D |
| G | No change, but formation of a little acid-albumin by the acid | No further change |
| H | As G | No further change |
| I | No change, except that fibrin was shrunken and a little alkali-albumin formed | No further change |

Another set of tubes, prepared similarly, confirmed these results. The medium in which the ferment acts most advantageously is therefore a weak acid, about equal to .2 per cent. HCl.

3. *What is the influence of alkalis and neutral salts on the ferment?*

The complete absence of any sign of activity in the alkaline tubes suggested, as the third point, an enquiry as to what had happened to the ferment in these; that is, had its activity been merely suspended, or had it been destroyed as is the case on similar treatment of pepsin?*. To determine this point, which had rather an important bearing on subsequent work, the two tubes D and F in the last series of experiments were made the subject of further investigation.

D contained the ferment in 1 per cent. Na_2CO_3 solution. It had been shown to be inoperative on fibrin in such a fluid. If its action were only suspended, it would be able to digest fibrin if the reaction were made acid to about the extent of '2 per cent. HCl. If it had been destroyed, such treatment would not restore the activity. At the same time, by the neutralisation of the Na_2CO_3 , some salt would be formed which might or might not have an influence on its rate of activity, should such exist.

The contents of the tube were therefore carefully neutralised, and the alkali-albumin referred to consequently precipitated. It seemed possible that any ferment present might be carried down by this precipitate, as such bodies do generally accompany precipitates in the fluid in which they are present. Only half the liquid was consequently filtered. Both were then added to an equal bulk of '4 per cent. HCl, and a fresh measure of fibrin added to each. The little quantity of the dissolved alkali-albumin in the unfiltered tube would not interfere with the result, as the activity was judged of by the disappearance or not of the fibrin. The filtered tube was labelled D_1 and the unfiltered one D_2 .

It was of course necessary to control the experiment by having two precisely similar tubes containing ferment that differed from these only in not having been exposed to the action of the alkali. It was necessary, therefore, to add to these exactly the same amount of neutral salt which D_1 and D_2 contained. This was done as follows:—

50 c.c. of Na_2CO_3 solution 1 per cent. were taken, and 5 c.c. dialysed ferment extract added. This reproduced the condition in D at commencement of the former experiment. Instead of allowing the alkali to act on the ferment, the mixture was at once made neutral. There was present now the same amount of salt as in D after digestion and subsequent neutralisation. The liquid was a little turbid from presence of a little proteid matter in the extract used. Half of it was filtered and half left unfiltered. 27·5 c.c. of '4 HCl and a measure of fibrin as before were now added to each; they were labelled D_3 and D_4 , and the whole set placed on a water bath.

The digestion was tested as it proceeded, but no marked results were obtainable for a longer time than usual. The digestion was allowed to proceed for 54 hours, when the following results were noted:—

* LANGLEY, 'Journ. of Physiol.,' vol. 3, p 246.

| | D ₁ | D ₂ | D ₃ | D ₄ |
|----------------|---|--|---|--|
| After 24 hours | No apparent change | No apparent change | Digestion beginning. Turbidity setting in. | Digestion beginning. About as D ₃ . |
| After 54 hours | No apparent change. Quantity of fibrin not lessened at all. Liquid quite clear. | No apparent change. (Unfortunately broke this tube in removing it, so did not measure the fibrin. To the eye there was no change.) | Liquid very turbid. About a quarter of the fibrin remained. | About one-fifth of the fibrin remained. |

From this set of experiments it becomes evident that alkalis destroy and do not merely suspend the active power of the ferment. From the slowness with which the digestion began, and the rate at which it proceeded, it seems that neutral salts in the solution have a deterrent effect upon it. Some previous experiments which I had made upon the behaviour of pepsin showed that it was similarly interfered with.

The experiments made upon the tube F were somewhat differently arranged. From the behaviour of the D set, the filtering seemed to make no difference to the result, so that it was omitted, but the control tubes were varied a little.

F contained the ferment in a .5 per cent. solution of Na_2CO_3 . It had been exposed to the action of the alkali for more than two days. As in the case of D, it was carefully neutralised and made up with HCl to a strength of .2 per cent. of the acid. A measure of fibrin as before was put into it, and it was labelled F₁.

The controls were prepared as under :—

To 50 c.c. of Na_2CO_3 .5 per cent., 5 c.c. of the dialysed ferment extract were added, and the whole neutralised at once.

Another 50 c.c. of Na_2CO_3 .5 per cent. were neutralised, and then 5 c.c. of the same ferment extract were added. Both were made up to .2 per cent. HCl, and a measure of fibrin placed in each. These were labelled respectively F₂ and F₃. All three were then placed on the water bath.

F₁ then contained the ferment extract that had been acted on for a time by the alkali, and some NaCl resulting from the neutralisation of the alkali.

F₂ contained the ferment that had been exposed to momentary contact only with alkali, and that at the ordinary temperature of the room; and the same amount of salt as F₁, similarly caused.

F₃ contained ferment that had not been exposed to even momentary contact with the alkali, and the same amount of salt as the others.

The subjoined Table gives the result of the action.

| Time of experiment | F ₁ | F ₂ | F ₃ |
|--------------------|---|--|---------------------------|
| hours 24 | No change apparent . . . | Digestion begun; more advanced than in D set of tubes after same interval, as shown by greater turbidity of liquid | As F ₂ . |
| 48 | A little change in the fibrin. Liquid turbid slightly. Fibrin measured seven-eighths of original bulk | Digestion advanced. About one-fifth of the fibrin only remaining | About as F ₂ . |

The comparison of this set with the D set confirms the view that the action of the ferment is retarded by salt, for, though slower than the usual rate of action, digestion was more rapid than in the D set. The action of the alkali on the ferment in the F set was distinctly deleterious, but the destruction was not complete as in the D set.

The outcome of these experiments gives then the following answer to the question stated :—

1st. The ferment resembles pepsin in being injured by action of alkalis upon it, the amount of injury depending upon the amount of alkali present.

2nd. The action is impeded by the presence of a small quantity of NaCl in the solution, the amount of hindrance being proportional to the amount of neutral salt present. The ferment is not destroyed by NaCl as it is by Na₂CO₃.

Further experiments showed that so long an exposure to the action of the alkali was not necessary for the destruction of the ferment power. In one case an exposure of three minutes rendered it inert.

4. *What is the condition in which the ferment exists in the resting seeds ; i.e., is it there as a zymogen or as a ferment ?*

The inability of v. GORUP-BESANEZ to show any ferment action in the Lupin extract, contrasting so strongly with the experiments described above, raised the question of the condition of the ferment in the resting seed as compared with the germinating one. In his paper already alluded to he does not mention the condition of his seeds in this respect, but the point suggested appears to throw some light on the reason of the maintenance of the long period of quiescence. If the ferment is present in the zymogen condition during this period, there seems no difficulty in understanding why germination is deferred, for it would not proceed until set up by a development of the active ferment from the zymogen.

The method adopted in examining this point was a modification of that recently described by LANGLEY and EDKINS* in their work on the condition of the ferment in

* 'Journ. of Physiol.,' vol. 7, pp. 371-415.

the cells of the gastric glands. It depends essentially on the destruction of the zymogen and of the ferment by alkalis, and by passing a stream of CO_2 through the extract of the gland. They find that, while both these reagents ultimately destroy both pepsinogen and pepsin, the destruction of the zymogen is much more rapid than that of the ferment by CO_2 , while the opposite conditions are found to obtain in the case of the alkali.

A quantity of the resting seeds was ground and freed from husks, and a watery extract made. To 20 c.c. of this, 2 c.c. of HCl of 1 per cent. strength were added, and the mixture warmed for an hour. This treatment would presumably convert any zymogen present into ferment, as it has that effect upon all the animal zymogens.

It was then carefully neutralised with .2 per cent. Na_2CO_3 , the quantity of the latter used being noted. For reference, this mixture may be called F.

To another 20 c.c. of the extract a mixture of 2 c.c. of 1 per cent. HCl and the same quantity of Na_2CO_3 that was used in the first case was at once added. This may be called Z. Both now contained the same amount of extract, and the same amount and description of salts. The only difference was that one had been warmed with acid and the other had not.

Now 5 c.c. of each of the mixtures F and Z so prepared were warmed for some time with 5 c.c. of 1 per cent. Na_2CO_3 . They were then again acidified, the strength eventually being .2 per cent. HCl , and fibrin was added. They were then put to digest in dialysers as before.

Control experiments were made by adding at once to 5 c.c. of each of the two mixtures F and Z the same amount of 1 per cent. Na_2CO_3 and of acid used in the previous cases, and these were then put to digest with fibrin in dialysers side by side with the others.

The only differences now between the last two sets of tubes were that the first two tubes had been warmed with alkali before being put to digest, while the last two had had the alkali neutralised without warming.

Simultaneous experiments were carried out on the effect of CO_2 on the extract. The gas was passed through equal quantities of the mixtures F and Z, and these were subjected to experiment with fibrin in dialysers, like the others. Controls were kept in which quantities of F and Z through which no CO_2 had been passed were tested. The dialysates were in all cases .2 per cent. HCl .

The experiments were repeated under varied conditions as to length of exposure to the action of these different reagents.

The result of the whole series was to show that the alkali had an injurious effect upon the extract always, but that the CO_2 did no harm if the latter had been treated with acid before the gas was passed through it.

Comparing these results with those of LANGLEY and EDKINS, the following conclusions seem probable :—

1. Zymogen and not ferment exists in the resting seeds.

2. This zymogen is readily converted into ferment by the action of dilute acids.
3. The zymogen is destroyed by the action of CO_2 , but the ferment is not.
4. Both zymogen and ferment are injured by the action of alkalis. There was not such a difference in the rate of injury as, from LANGLEY and EDKINS's results on pepsinogen, I expected to find.

The conditions of germination, and the preliminary changes which take place at its onset and really start it, may be deduced from these considerations. The reaction of the resting seed is neutral, and the contents of the cells are dry. On the admission of water to the cells, which invariably precedes germination, the reaction rapidly becomes acid, probably by certain decompositions made to take place in the protoplasm, whereby vegetable acids are formed. This seems not an unreasonable hypothesis, as the rapid formation of such acids in turgid cells is the only way in which the continual turgescence can be maintained, the latter depending on the osmotic equivalent of the acid. This acid, reacting on the zymogen present in the cells, developes from it the ferment, under the action of which the transformation of the resting products can readily take place.

5. *Action of the ferment on the proteids of the seed.*

The existence of the ferment and certain of the features of its action thus having been ascertained, it remained to investigate the result of its working on the proteid bodies existing in the seed. According to VINES,* these consist in the Lupin of a mixture of hemialbumose and a vegetable globulin. From the recent researches of KÜHNE and CHITTENDEN,† the body hitherto called hemialbumose by them appears to be a mixture of, in many cases, four distinct albumoses. I had not time, unfortunately, to submit VINES's hemialbumose to very elaborate investigation, but I was able to see that it is composed of at least two albumoses, which correspond fairly well with KÜHNE's protalbumose and heteroalbumose.

A watery extract of the ground resting seeds was boiled to separate the globulin which had dissolved by the assistance of the inorganic salts in the seed; it was then filtered and submitted to dialysis. None of the albumose passed the dialyser even after more than a week's exposure; the dialysate gave no biuret reaction, and a faint opalescence only with excess of alcohol. A trace of sugar passed through, but not more than the merest trace. After twenty-four hours' exposure I found that the removal of the salts had caused a copious precipitate to fall, while the solution, freed from this precipitate, gave a further precipitate on addition of HNO_3 . A little of the boiled original extract, containing, as noted, a small quantity of inorganic salts, gave a fairly good precipitate on large dilution with water. All these precipitates behaved like albumoses in that they were dissolved on heating the solution in which they were suspended, and came down again as the liquid cooled.

* *Op. cit.*

† *Op. cit.*

A 10 per cent. NaCl extract of the seeds was found to give similar reactions, but to contain more of the body precipitable by dialysis or dilution of its solution. There was also extracted by this fluid a greater quantity of the globulin, as shown by the greater amount of coagulum produced by boiling.

As already mentioned, very careful and long-continued dialysis showed that neither of the proteids of the seed was capable of dialysis. The dialysate of the boiled watery extract, on concentration and slow evaporation on a glass slide, showed only here and there a needle-shaped crystal. The seed hence contains, before germination begins, two albumoses (protalbumose and heteroalbumose) and a globulin, but no peptone, nor any crystalline derivative of proteids.

Before discussing the behaviour of the ferment when mixed with these bodies for purposes of experiment, it may be well to mention certain changes which took place in a watery extract of the germinating seeds, prepared at the same time as the glycerine one used in the experiments already detailed. This watery extract when first made was turbid, and would not filter clear. It contained albumoses, as evinced by a precipitate given on addition of HNO_3 or acetic acid. After standing some days at the temperature of the laboratory, the turbidity had partially disappeared, and addition of acid failed to produce a precipitate. The ferment extracted by the water from the germinating seed had evidently performed a process of digestion of the proteids extracted coincidentally with itself. A quantity of it was then submitted to dialysis for some days, and the dialysate gave a slight biuret reaction, much masked by a colouring matter that diffused out; when concentrated and evaporated on a slide, it slowly deposited crystals resembling those of asparagin.

The composition of the stored proteids having been ascertained, a quantity of the mixed albumoses was prepared from the salt extract of the resting seeds. This was chosen, as it seemed to contain the two albumoses more in the proportion in which they existed in the seed than did the watery one. The heteroalbumose is insoluble in water, and so only so much of it dissolved in the extract as was enabled to do so by the small amount of salts in the seed. This salt extract was boiled to separate the globulin, which coagulated and was filtered off. The liquid was then completely precipitated by acetic acid, and the precipitate separated by decantation and subsequent filtration, washed, and dried. It was then dissolved again and precipitated by alcohol. After standing under this for some time, it was dried and used for the purposes of the experiment, being mixed with a quantity of the ferment in new tested dialysers. The solution was made acid to an amount equal to .2 per cent. HCl , and outside the dialysers acid of the same strength was used. The relative quantities of the two albumoses used in different experiments varied considerably, but neither was absolutely isolated in any. In all the experiments the course of the digestion was the same, and a mixture of both albumoses with the globulin behaved in the same way.

The first body formed during the digestion of the albumoses was an acid-albumin, exactly like the parapeptone described as occurring when the ferment was acting on

fibrin. This was precipitated on neutralisation, and was soluble in weak acids and alkalis. No doubt part of it was due to the action of the acid present, for a control tube, with acid only and no ferment, showed the formation of some, though not so much. The appearance of this body always struck me as peculiar, as, according to KUHNÉ's theory of proteolysis, hemialbumose is not an antecedent of such a body, but is converted at once into peptone. To this point I shall return later, merely noting here that the occurrence of the acid-albumin made me particular to see that in later experiments there was no proteid subjected to the action of the ferment but the albumoses, and then, as before, I always found this neutralisation precipitate present.

Shortly after this body had appeared the existence of true peptone was recognisable in the dialysate by the biuret reaction. I never found the dialysates give this unless ferment was present in the digesting mixture. The HCl alone had the power of producing the acid-albumin, but could take the digestion no further, or at most could produce only the merest trace of peptone.

In many cases there was mixed with the solution of the albumoses a small amount of a peculiar colouring matter, which, on the addition of caustic soda, gave a strongly marked yellowish-brown tinge to the liquid. The biuret reaction was consequently not very easily noted. I therefore adopted another test for peptone, which I found to be exceedingly delicate.* It consists in freeing the solution from all other proteids by boiling with freshly prepared ferric acetate and then adding to it acetic acid and a drop of metatungstic acid. Any peptone, which may be present, is thereby precipitated in a finely granular form. By this reaction I was able to find traces of peptone which were not discoverable by the biuret reaction, for the reason already stated. I tested the controls always by this method, at the same time as the dialysates of the proteids with ferment present, and found, as indicated by the biuret, where practicable to apply this, that a large quantity of peptone was formed by the ferment, and the merest trace only by the HCl.

With much more trouble I succeeded in showing that the ferment produced crystalline bodies, showing crystals similar to those already alluded to as being formed during the digestion of fibrin. In some cases these were mixed with other crystals, resembling those of asparagin, and in yet other cases the latter were by far the most numerous. These were deposited, on concentration of the dialysates and evaporation on glass slides. There was a difficulty in satisfying myself for some time that the crystals really came from the proteids in consequence of digestion, as my ferment extracts, being glycerine extracts of the germinating seeds, might have contained some of these bodies, although they had been themselves dialysed. Several experiments were therefore conducted to settle the point. In one series of these the dialysates were changed several times and the quantity of crystals obtainable from each observed. The fifth dialysate contained them in greater quantity than the first, which indicated a formation of them as the digestion proceeded, for if they were in the

* MARTIN: *Op. cit.*

extract, to start with, they must have gradually diminished in each change. The most satisfactory experiment was one that was conducted on rather a large scale. A quantity of the solution of the mixed albumoses, prepared and purified as before described, was taken and acidified up to .2 per cent. HCl, and 25 c.c. of the glycerine ferment extract were added to it. The quantity in all was 300 c.c. This was digested for a week in a large beaker on a water-bath kept constantly at about 40° C. After the expiration of this time 100 c.c. of the fluid were taken from the beaker and boiled. On neutralising it, as before, a copious precipitate fell, which was filtered off. This contained the acid-albumin formed and part of the undigested albumoses. A further quantity of these came down on addition of ammonia, and these in turn were filtered off. The clear liquid was treated then according to V. GORUP-BESANEZ's directions to separate the crystalline amides, as has already been described in the case of the fibrin digestion. The leucin, &c., was precipitated by basic acetate of lead, the lead compound, after washing, decomposed by SH_2 , and the filtrate from this concentrated to very small bulk, when it deposited the crystals in abundance. From the precautions taken, and the amount of them obtained, these could have had no other origin than the digestion of the albumoses.

Besides these experiments made on the albumoses alone, some were carried out on whole extract, containing the globulin in addition. The course of the digestion, the disappearance of the original proteids, and the several products formed were the same in all cases.

The action of the ferment then upon the proteids in the seed was marked by the successive formation of acid-albumin or parapeptone, peptone, and leucin and asparagin in different proportions.

6. *Comparison of these results with the changes ascertainable by examination of the seed and seedling during germination.*

Following out these experiments, which were all performed apart from the seed itself, it seemed advisable to ascertain how far these successive steps could be recognised in the germinating seeds, and hence to deduce, if possible, the form in which the nitrogenous matter travels in the seedling. In his work already quoted VINES calls attention to the difference between the composition of the cell contents in the radicles and in the cotyledons of the germinating seeds, showing that when the germination took place in the dark, and the young plant was in consequence etiolated, there was much albumose and little asparagin in the cotyledons, while in the radicles asparagin alone was found. V. GORUP-BESANEZ also has stated that in absence of light he found leucin and asparagin in the shoots of the Vetch.

The composition of the resting seed has already been described. When germination had just begun, and the radicle was just protruding, the cotyledons were found to contain a considerable quantity of acid-albumin, but not much peptone. Seeds rather

further advanced contained more of the latter, and asparagin was also present, though in small amount. The radicles showed the absence of peptone. With metatungstic acid and acetic acid there was hardly any opalescence. When the extract of the ground-up radicles was concentrated and left in an evaporating dish, large rhombic crystals of asparagin separated out in considerable quantity. These results confirm, then, those arrived at by acting on the proteids in the laboratory. The changes in germination following the development of the ferment appear to be—

1. The formation successively of acid-albumin or parapeptone, peptone, and crystalline amides.

2. The travelling of the latter bodies to the points where plastic material is required.

It appears certain that the nitrogenous crystalline bodies are not formed at first, but that at least two intermediate bodies precede them. Also that the peptone formed is but a stage in this production, and does not leave the seat of its formation. Hence peptone is not the form in which plastic nitrogenous material travels in the seedling.

The histological changes accompanying these chemical ones are interesting. In the resting seed the proteid matter is present in the form of aleurone grains, which are small round bodies embedded in a network of protoplasm. Their outlines are sharp, and they occupy about half the whole space of the cell. In a seed in which germination has just commenced the grains are found to have become larger, probably from taking up water, and the protoplasmic network is consequently more compressed. A later stage shows the grains to have a much less distinct outline, though they retain their almost spherical shape. They are now studded with sparse granules, and appear to be dissolving from within outwards. When the radicle has attained a length of about 1·5 inch the disintegration of the grains is very marked, and the protoplasm contains empty spaces in which they formerly lay. In the actively growing seed, the radicle being 2 to 3 inches long, there is little left except the meshwork of protoplasm which, now relieved from tension, is seen to be very loose, and to contain large vacuoles.

AMYLOLYTIC FERMENTS.

The manner in which the stored-up cellulose is made use of in the plant has not been hitherto shown. Beyond the fact that in some seeds there is a large accumulation of it, and that then no starch appears in the reserve materials, but little is known. Judging from analogy with both starch and proteids, there seems some likelihood of the action being a ferment action.

Some seeds of the Date (*Phoenix dactylifera*) were germinated for me by Mr. LYNCH at the Botanical Gardens, Cambridge. When the young plants had attained a height of about six inches they were removed, and the now partially softened seed submitted

to investigation. In his 'Text-book of Botany,' SACHS has figured the seed of *Phoenix* in the several stages of germination. From his drawings it appears that the young plant remains attached to the seed by part of the cotyledon, and by means of this it avails itself of the stored-up matter. On making a transverse section of the seed, it was seen to consist of two parts, the hard endosperm, and the soft cotyledon partly surrounded by the former. The endosperm was composed of elongated oblong cells, with enormously thickened walls, containing a very small amount of protoplasm and no starch. The cotyledon was made up of rather loosely arranged cells, containing starch granules and a very distinctive epidermis. This was composed of square cells, cuticularised, and containing a very granular deeply-staining protoplasm, but no starch.

The cotyledon was separated from the hard parts, and glycerine extracts were made of both, each being ground up as finely as possible.

The extracts were in the first instance put to digest with some ground resting seed, but after prolonged action neither of them seemed to have any effect; at least the quantity of ground cellulose had not apparently diminished.

The extract of the cotyledons was next tried on a small piece of manufactured cotton. A similar quantity of the extract was well boiled, and to it a similar piece of cotton added. After two days' digestion the former piece appeared to be attacked at the edges, where threads had frayed out. Both were now removed and boiled in FEHLING'S fluid. The one which had been in the unboiled tube became coloured with reduced copper oxide in patches, particularly in the parts which seemed softest, but the other underwent no change of colour at all. There was apparently in the extract a very small trace of a ferment which produced sugar from the cellulose, but so very little in amount as to lead to the view that the change of the cellulose in germination was not due to an isolable ferment. On looking at the cotyledon, and noticing the character of the cells covering it, which are consequently in contact with the cellulose of the endosperm, it appears more likely that the latter is gradually eaten away by this epidermis, and that the ferment only exists in these cells. This seems probable, too, from a section of the endosperm. The cells are seen to be gradually broken down where they are in contact with the cotyledon, the other cells remaining intact till they are reached by the latter.

There was no evidence that the extract of the endosperm had any action on cellulose at all. Evidently in it there is no ferment, and the change is due to agencies quite external to its cells.

Neither extract had any diastatic action.

I can confirm previous writers as to a diastatic ferment in the Lupin seeds, besides the proteolytic one.

I may summarise my results on germinating seeds as under :—

1. There exists in the Lupin seed a proteolytic ferment, working in an acid medium, and capable of converting fibrin into peptone, leucin, and tyrosin.

2. This ferment exists in the resting seed in the form of zymogen, and the latter very readily, by the action of acids, is transformed into active ferment.

3. Its action is much interfered with by presence of too much neutral salt, and it is quite destroyed by the action of alkalies.

4. It works with extreme slowness.

5. Its action is much like that of pancreatic juice, but more hemialbumose is formed by it than by the latter.

6. It acts on the proteids in the plant in such a way as to convert them into peptone, and then into leucin, asparagin, &c.

7. The nitrogenous plastic material travels to the growing points in the form of these amide bodies, and not in that of peptone.

8. The conversion of cellulose in the date seed into plastic material is not carried out by an isolable ferment in the endosperm, but by the gradual breaking down of the latter, brought about by the epidermal cells of the cotyledon, which contain the ferment in small quantity.

The proteolytic ferment of the Lupin has considerable importance from the slowness of its action, which seems as if it would afford facility for the examination in detail of the course of proteolysis. In the foregoing paper I have called attention to two points that seem to offer a field for further investigation. The first of these is connected with the apparent formation by this ferment of considerably more "hemialbumose" from fibrin than is formed from it by either pepsin or trypsin. Whether hemialbumose be a single body, or more probably a mixture of several, it appears to approach the globulins in its reactions more nearly than does the acid-albumin or parapeptone, which is formed coincidently or later. Thus, like them, it is more freely soluble in dilute salt solutions than it is in water, in which fluid globulins are altogether insoluble. From its solution it is precipitated on saturation with the same neutral salt; at least part of it is precipitated by dialysis or dilution, just as most globulins are. In its behaviour to heat it begins to show a difference. Both globulins and hemialbumose form opalescent solutions in the cold; on raising to a temperature above 70° C., the globulins are coagulated; the hemialbumose goes into solution completely, being again thrown down on cooling. This difference is carried a step further with parapeptone, for when in solution it is quite unaffected by heating or cooling. There is, however, a difference between the latter two bodies as to the liquid in which they are soluble. In the peptones the solubility at all temperatures is still seen, and the necessity for acid or alkaline reaction in the fluid to secure such solubility has disappeared. The point suggested is, therefore, whether hemialbumose is really the first product formed, and whether it gives rise later to the other products seen, viz., parapeptone and peptone. If so, the greater quantity of it found with the

Lupin ferment may be caused by the slowness of action of the latter allowing it to accumulate before the process can be carried further.

The next point I wish to call attention to is the formation of acid-albumin or para-peptone in the digestive process brought about by the Lupin ferment in a solution of albumoses. Whether these are identical with KÜHNE's hemialbumose or not, they give reactions showing a very close connexion with it, as pointed out by VINES.* The acid-albumin formed closely resembles MEISSNER's para-peptone, and is hardly distinguishable from KÜHNE's "antialbumose." According to the theory of proteolysis advanced by the latter observer, the first decomposition of such a proteid as albumin or fibrin is the splitting of it into two groups of bodies, which he calls an "anti-" and a "hemi-" group, to each of which belongs an albumose and a peptone, and these two groups seem in their further decomposition to be independent of each other. Neither should, therefore, give rise to a member of the other group. Now para-peptone, closely resembling antialbumose, is a member of the "anti-" group, hemialbumose a member of the "hemi-" group. Consequently, if KÜHNE's theory be true, hemialbumose, when present alone, should give rise at once to peptone, and antialbumose or para-peptone should not appear.

The experiments I have narrated do not of course settle the point, for, to be quite sure, it is essential that no member of the "anti-" group should be present. Though the albumoses I worked with were carefully prepared, it seems necessary that more should be learnt about their nature and reactions, and the best way of isolating them absolutely pure, before any dogmatic statement should be made. The experiments suggest an enquiry into the fate of such perfectly isolated albumoses when acted on by trypsin, pepsin, and the Lupin ferment. Such an enquiry should embrace the animal albumoses as prepared from fibrin, and the vegetable ones that can be obtained from the seeds of plants.

Should such occurrence of para-peptone from pure albumoses be found, the point would have an important bearing on the question I raised just now as to the relative places of hemialbumose and para-peptone in the course of proteolysis.

To these questions I hope to return at a subsequent period.

* *Op. cit.*

IV. *The Carbonic Acid, Organic Matter, and Micro-organisms in Air, more especially of Dwellings and Schools.*

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[PLATE 6.]

It has been fully proved that the habitual breathing of air vitiated by the presence of human beings has a most important effect on the death-rate. We nevertheless possess as yet scarcely any accurate data as to the connection between the death-rate and the quantities of such impurities as it is at present possible to measure. Nor, so far as we are aware, have any systematic observations been made as to the relative amounts of these impurities in different samples of vitiated air.

The quantity of carbonic acid (or of carbonic acid and oxygen in more refined investigations) is usually taken as a measure of the total impurity. It is, however, highly improbable that an increase in the carbonic acid and a slight diminution in oxygen materially affect the death-rate (cf. HIRT, 'Die Krankheiten der Arbeiter,' 1873).

The organic matter and micro-organisms in the air are in all probability far more important factors.

The original object of the present investigation was to examine systematically the air of various classes of houses with the view of comparing the amounts of carbonic acid, organic matter, and micro-organisms with one another, and with the death-rates in the same classes of houses. During the progress of our work, however, several additional questions presented themselves, and the actual scope of the research has therefore been as follows :—

1. An investigation of the outside air so far as was necessary for purposes of comparison with the determinations made inside buildings.

2. A comparison of the quantities of the above-mentioned constituents of the air in one-roomed, two-roomed, and the better class of houses with one another, and with the death-rates and relative frequencies of various diseases in the several classes of dwellings.

3. An examination of the air in schools, with special reference to the cubic space

and to different methods of ventilation, and other circumstances affecting the quality of the air.

4. An examination of the air in some other classes of buildings.

5. An investigation into the sources of the organic matter and micro-organisms of air inside buildings, and the circumstances affecting the number of micro-organisms; also of the relative number of bacteria and moulds in both outside and inside air.

METHODS.

1. *Carbonic Acid*.—PETTENKOFER'S well-known method was employed.

2. *Organic Matter*.—The process described by Professor CARNELLEY and Mr. MACKIE was made use of ('Roy. Soc. Proc.,' vol. 41, p. 238).

It must not be forgotten that the term "organic matter" is a very indefinite one, and that by it is really meant the bleaching action of the air on a dilute solution of potassium permanganate acidified with sulphuric acid. It therefore includes not only organic matter properly so called, but those substances which air sometimes contains, such as sulphuretted hydrogen, sulphurous acid, &c., which also bleach a permanganate solution. Even the organic matter itself may be of very different kinds and vary considerably as regards its influence upon health, some doubtless being quite harmless, whilst some may exert a very deadly effect. In so far, therefore, as the method does not distinguish between these various constituents of air, but brings them all into the same category, it is a very imperfect method. But, as no better process has yet been devised, it is the only one which has been at our disposal.

3. *Micro-Organisms*.—In determining the number of micro-organisms, HESSE'S method ('Mittheilungen aus dem Kaiserlichen Gesundheitsamte,' vol. 2, p. 182) was employed.*

A piece of wide glass tubing, about 70 cm. long and 3.5 cm. wide, is closed at one end by means of a perforated india-rubber cork, through which passes a small piece of glass tubing about 10 cm. long and 1 cm. wide, and containing a plug of cotton wool at each end. The other end of the large tube is closed by two india-rubber caps fitting one over the top of the other, the inner one being perforated by a round opening of about 1 cm. in diameter. The whole being thoroughly cleansed, about 50 c. c. of KOCH'S jelly (containing the juice of 1 lb. of meat, 10 grammes of peptone, 5 grammes of common salt, and 50–100 grammes of French leaf gelatine to 1 litre of water, and, after heating, very slightly over-neutralised with sodium carbonate) is introduced into the tube. The tube, with its contents, after replacement of the cork, is exposed to a temperature of 100 C., in an atmosphere of steam, for at least an hour, and then allowed to cool. When the jelly assumes a syrupy consistency the tube is

* As HESSE'S method is not generally known, especially among chemists, and as the literature referring to it is not easily accessible, it has been thought advisable to describe it in greater detail than would otherwise be necessary.

placed in a horizontal position and turned round until the jelly is just beginning to set; the whole of the inner surface of the tube is by this means coated with jelly, and a flat surface is formed at the bottom, where most of the jelly collects before setting. When the tube is to be used it is set horizontally on a tripod, or other convenient stand, with the flat surface of jelly on the lower side of the tube. The outer cap is then removed, and a measured quantity of air drawn slowly through the aperture in the inner cap and along the tube by means of an aspirator, the india-rubber tube of which is connected with the end of the small tube passing through the cork of the large tube. For aspirating, two graduated bottles are used, one being hung to the upper part of the tripod, and the other standing on the ground. The rate of flow is regulated by the size of the glass nozzle through which the water flows into the lower bottle, or by means of a small screw clip.

The whole of the micro-organisms in the aspirated air always settle on the lower half of the tube; and when a proper rate of flow is chosen the resulting colonies (which develop in the course of a few days) appear closest near the cap, gradually becoming more and more sparse as the farther end of the tube is approached, so that the last part of the tube is entirely free from colonies (see Plate 6, figs. 2-5). For further information we must refer to HESSE's paper; but it is desirable to mention more specially some details of the method as it was employed by us. As regards length and width of tubes, we did not consider it necessary in our experiments to adhere strictly to one size. It is exceedingly difficult and expensive to procure tubing of the same width and in such lengths as will cut up without great waste into pieces of say 70 cm. long; and for our purpose the advantages would have been scarcely appreciable.

HESSE recommends that the tubes should have edges turned out at one end, in order that the caps may fit more tightly. This, however, increases the risk of cracking during sterilisation, and makes it difficult to remove or replace the cap without a good deal of inconvenient manipulation, which increases the danger of artificial infection of the air. We found it better to employ square pieces of india-rubber sheeting, which were fastened by means of stout umbrella rings. The inner cap is first fixed on, the part of the cap next the end of the tube being freed from creases by slightly stretching the cap. It is then easy to fix the outer cap by means of a ring applied quite close to the end of the tube. If any creases are left under the ring the tube is sure to leak. An additional ring applied higher up keeps the edges of the square in proper position. When the tube is used the last-mentioned two rings are removed, and the outer cap can then be taken off without any manipulation likely to infect the air. During the experiment the cap is placed in a solution of corrosive sublimate and replaced still moist, the superfluous drops having, however, been first carefully shaken off.

The apparatus used for sterilisation was essentially the same as that employed by HESSE, and consisted of a sufficiently large cylinder of iron plate, about $3\frac{1}{2}$ feet long, fitted on the top of an ordinary potato-steamer and provided with a perforated lid.

The whole was covered externally with felt. A temperature of 100° was easily maintained inside the cylinder.

The tubes were at first sterilised by being kept for half an hour at 100° on two successive days. Latterly they were simply kept for an hour at 100°. Any failure in sterilisation would be easily detected by the appearance of colonies inside the jelly, and by their distribution round the tube. But this was never obtained in the case of tubes sterilised by the above method (cf. KOCH, GAFFKY, and LÖFFLER, 'Mitth. a. d. K. Gesundheitsamte,' vol. 1, p. 332 *et seq.*), nor did any colonies ever appear inside sterilised tubes kept unused for long periods.

The rate of air-flow employed was, as a rule, about one litre in three minutes. The most suitable rate in any given case depends on the number of micro-organisms in the air and the rapidity with which they settle. When there are many in the air to be examined it is best, as a rule, to make the rate of flow more rapid. The error arising from the crowding together of colonies near the entrance is far greater than any likely to occur from the passage of micro-organisms into the cotton wool. Not only do the colonies coalesce if crowded together near the entrance, but substances appear to become diffused through the jelly, which entirely prevent the growth of some of them. Again, when the particles to which bacteria are attached are heavy, as occurs, for instance, in outside air on a dry windy day in towns, a rapid current is also necessary on account of the heavy particles coming down very rapidly on entering the tube. On the other hand, a slower current is desirable when there are no heavy particles in the air, as in the case of a room which has been standing quiet, or outside air in damp weather. It is, of course, quite easy to tell from the distribution of the colonies in the tube whether a proper rate of flow has been chosen.

The quantity of air taken was usually half a litre in bad atmospheres, one or two litres in better ventilated rooms, and from one to ten litres in outside air, according to circumstances. The latter quantity would frequently be insufficient in making systematic observations on outside air, as in many cases no colonies develop from ten litres. The quantities taken were, however, sufficient as regards the purposes we had in view in examining outside air. The tubes after the samples had been collected were exposed horizontally on racks in a room the temperature of which was kept tolerably constant during the day all winter by means of a warm-air ventilating apparatus. They were left until no more colonies made their appearance—a period of, practically speaking, from three to four weeks. In a good many cases the colonies had begun to run together before this time had elapsed. In such cases we made an allowance for probable increase, based on the percentage increase observed in other tubes.

Probably a better arrangement would have been to have kept them in a large incubator at about 20°, but, having begun by exposing them in the laboratory, we thought it best to continue doing so all through our experiments.

The tripod employed was about three feet high, so that the samples were always collected at that height from the floor.

As some adverse criticisms of HESSE's method, contained in an article by PAWLOWSKY ('Berliner Klinische Wochenschrift,' 1885, No. 21), appear to have attained some notice, it may be well to state that these criticisms, as will easily be seen on reading the article, depend on a complete misunderstanding of the method of sterilisation employed by HESSE. The new method suggested by PAWLOWSKY is not only no improvement, but would give absolutely nugatory results.

We have preferred HESSE's method on account of its great simplicity and other important advantages. We consider it to be a most convenient and elegant process. MIQUEL's method ('Organismes Vivants de l'Atmosphère,' p. 175) could not well have been applied by us under the conditions to which our work was subject. MIQUEL (p. 151) used as a standard nutrient medium a *bouillon* of LIEBIG's extract, of 1·024 specific gravity, and incubated it at 30° to 35°. How such a nutrient medium compares with KOCH's meat jelly, used under the conditions of our experiments, has not, so far as we know, as yet been determined. We cannot, therefore, compare at present the numerical results of our observations with those of MIQUEL in his valuable series of observations on the micro-organisms of outside air.

In stating our results, the carbonic acid is represented in all cases by the number of the volumes of the gas contained in 10,000 volumes of air.

The organic matter is represented by the volumes of oxygen required to oxidise the oxidisable organic matter in 1,000,000 volumes of air.

The micro-organisms are represented by the number per litre of air. It must also be distinctly understood that this number includes only those which grow on KOCH's nutrient jelly of the composition previously stated, and kept in a room under ordinary conditions. If the jelly, for instance, were acid, the numbers and the ratio of bacteria to moulds would be different.

OUTSIDE AIR.

In order to draw conclusions from an examination of air inside buildings, it is of course necessary to know the state of the outside air. As regards each of the constituents estimated, considerable variations were found at different times and places.

The following Table gives the results of determinations made on different days and in all kinds of weather during the winter and spring of 1885-86, and in various parts of the towns of Dundee and Perth. The observations were made at intervals over the same period as that during which the air of schools, houses, &c., was examined.

| | Dundee (town, quiet places.) | | | | Dundee (suburbs.) | | | | Perth (outskirts). | | | |
|--------------------------|------------------------------|--------|---------|-------|------------------------|--------|---------|------|------------------------|--------|---------|-------|
| | No. of determinations. | Lowest | Highest | Mean. | No. of determinations. | Lowest | Highest | Mean | No. of determinations. | Lowest | Highest | Mean. |
| Carbonic acid . | 32 | 2.2 | 5.6 | 3.9 | 5 | 1.8 | 3.5 | 2.8 | 3 | 2.9 | 3.5 | 3.1 |
| Organic matter . | 46 | 1.7 | 16.8 | 8.9 | 10 | 0 | 5.6 | 2.8 | 5 | 1.7 | 4.5 | 3.1 |
| Total micro-organisms —* | 15 | 0 | 2.2 | 0.8 | 1 | .. | .. | 0.1 | .. | .. | .. | .. |
| Bacteria . | 14 | 0 | 1.9 | 0.6 | 1 | .. | .. | 0 | .. | .. | .. | .. |
| Moulds . | 14 | 0 | 1.3 | 0.2 | 1 | .. | .. | 0.1 | .. | .. | .. | .. |

The above Table shows :—(1) That the average carbonic acid and organic matter were much lower in suburban than in town air. Presumably they would be still less in country air. No conclusion can be drawn as regards the relative proportion of micro-organisms in town and suburban air, as only one determination was made in the latter case. (2) That this difference is relatively much greater in the case of the organic matter than in that of the carbonic acid. (3) That the quantity of organic matter, at least in town air, varies within much greater limits than that of the carbonic acid. (4) That the average number of micro-organisms in town air was rather less than one per litre for town air in Dundee in winter. (5) That the number of bacteria in the air of quiet places in winter in Dundee was about three times as great as the number of moulds. In the open streets during dry and windy weather the proportion of bacteria is much greater (see pages 99 (Table) and 101).

The numbers of which the above are the means exhibited variations of every degree within the limits shown in the Table. Some of the causes of these variations will appear from the following Tables.

Influence of Day and Night, and of Open and Closed Places.

This is clearly shown in the Tables given below. The day has been supposed to extend from 5 A.M. to 7 P.M., as these are about the hours at which work begins and ends at the mills and factories in Dundee. Although the ordinary work begins at 6 A.M., the boiler fires, &c., begin to smoke about 5 A.M. By "open places" we mean ordinary streets, large yards, squares, &c.; whilst by "close places" are meant narrow lanes, entries, back-yards, and other places closely surrounded by houses, and more especially by crowded low-class houses. No determinations were made in close places during the day.

* A larger number of micro-organisms (11 per litre on an average) were found at places in or close to open streets on which there was much traffic (see page 99, Table). These observations were made in April and May, 1896, and subsequently to those detailed above.

All the observations included in the above Table were made with ten litres of air.

| | Open places Day | | | | Open places Night | | | | Close places Night. | | | |
|-------------------------|-------------------------------|---------|----------|------|-------------------------------|---------|----------|-------|-------------------------------|---------|----------|------|
| | No of determi- nations. | Lowest. | Highest. | Mean | No of determi- nations. | Lowest. | Highest. | Mean. | No. of determi- nations | Lowest. | Highest. | Mean |
| Carbonic acid . | 20 | 2.2 | 5.3 | 3.8 | 9 | 3.0 | 5.6 | 4.1 | 7 | 3.2 | 5.4 | 4.2 |
| Organic matter . | 34 | 1.1 | 16.8 | 9.5 | 9 | 2.2 | 6.7 | 3.9 | 7 | 5.8 | 11.8 | 8.4 |
| Total micro-organisms — | 6 | 0.3 | 2.2 | 1.2 | 2 | 0.1 | 0.2 | 0.15 | 6 | 0 | 1.3 | 0.54 |
| Bacteria . . | 6 | 0 | 1.9 | 0.7 | 2 | 0.1 | 0.2 | 0.15 | 5 | 0 | 1.3 | 0.49 |
| Moulds . . | 6 | 0.2 | 1.3 | 0.5 | 2 | 0 | 0 | 0 | 5 | 0 | 0.1 | 0.01 |

The above Table shows: (1) That, with the exception of the carbonic acid, all the determinations made in open places gave much lower results during the night than during the day. The organic matter was reduced to less than one-half, and the micro-organisms to nearly one-tenth, of what they were during the day. The carbonic acid, though represented as being slightly less during the day, was practically the same; for, according to the method of treating the data, the night would show a slightly higher or a slightly lower result than the day. (2) That in all cases the determinations made during the night gave higher results for close than for open places. With the exception of the carbonic acid, this difference is very considerable. Though the figures for carbonic acid are near, yet the average for close is distinctly higher than for open places. The average for open places is possibly a little too high, as any other method of treating the data would give a somewhat lower result. (3) That, with the exception of the carbonic acid, even close places at night give a lower average than open places by day, thus proving distinctly that both organic matter and micro-organisms are considerably less during the night than during the day. The moulds more particularly showed a large reduction.

The influence of day and night, and the effect of town and suburban conditions, is shown in a conclusive manner by the next Table. This Table records a series of hourly observations made simultaneously at two different places for a period of twenty-four hours. One of the places at which the samples were collected was the large open playground of the Dundee High School in the centre of the town, and the other a somewhat elevated position on the western outskirts overlooking the Tay. The prevalent direction of the wind was about south-west, *i.e.*, across and down the valley of the Tay from the Ochil Hills. The standard solution employed in both cases was the same; and the analyses, apart from the collection of the samples, were carried out by the same person. The results of the full Table (p. 68) may be summarised as follows:—

| April 2nd and 3rd, 1886. | | Town. | | | | West suburbs. | | | |
|----------------------------|---------------------|-----------------------|---------|----------|-------|------------------------|----------------------------|----------|-------|
| | | No of Determinations. | Lowest. | Highest. | Mean. | No. of Determinations. | Lowest. | Highest. | Mean. |
| Day, 5 A.M. to 7 P.M. | Carbonic acid . . . | 18 | 2.6 | 4.1 | 3.25 | 18 | 1.7 | 2.8 | 2.3 |
| | Organic matter. . . | 12 | 2.8 | 9.6 | 6.2 | 18 | 1.7 | 8.1 | 5.8 |
| Night, 7 P.M. to 5 A.M. | Carbonic acid . . . | 7 | 3.0 | 4.1 | 3.45 | 10 | 1.7 | 2.4 | 1.75 |
| | Organic matter. . . | 6 | 2.5 | 6.8 | 4.6 | 10 | { Too small to estimate. } | | 1.86 |

As before, the day is taken as extending from 5 A.M. to 7 P.M.

It will be seen that the quantity of carbonic acid in the air during the day on which the experiments were made was extremely low, even falling to 1.7 volumes on the west of the town during the night. The frequent lowness of the carbonic acid in Dundee during last winter is very remarkable, and difficult to account for. It is true that the results were obtained during winter, and many of them in the middle of the

| April 2nd and 3rd, 1886. | Temperature. | | Carbonic acid. | | Organic matter. | | Remarks. |
|-----------------------------|--------------|------------------|----------------|------------------|-----------------|------------------|---|
| | Town. | West suburbs. | Town. | West suburbs. | Town. | West suburbs. | |
| | ° Fahr. | ° Fahr. | | | | | |
| 7.15 A.M. | .. | .. | .. | .. | 5.8 | 8.1 | { Somewhat overclouded, otherwise bright sun- shine. Breezy, S.S.E. Chimneys smoking strongly. Smell of smoke. Smoke also blown across to west of town from bridge works. |
| 8.15 | .. | .. | 2.7 | 1.9 | 8.1 | 4.1 | |
| 9.15 | 50 | 48 | 2.6 | 2.4 | 4.5 | 6.3 | Sunny, S.S.E. |
| 10.15 | .. | 49 | 2.9 | 2.6 | .. | 6.3 | Overcast; strong wind, S. |
| 11.15 | 52 | 49 | 3.3 | 2.6 | 6.5 | 4.8 | Ditto, S.S.W. |
| 12.15 P.M. | .. | 49 | 3.2 | 2.8 | 3.2 | 1.7 | Ditto. |
| 1.15 | 53 | 51 | 4.1 | 2.6 | .. | 4.8 | Ditto. Wind rather more from West. |
| 2.15 | 54 | 49 | 3.3 | 2.2 | 7.1 | 7.0 | Ditto. Wind S.W. |
| 3.15 | .. | 48 | 2.9 | 2.4 | 9.6 | 5.6 | Ditto. |
| 4.15 | 49 | 46 | 3.2 | 2.2 | 2.9 | 5.8 | Rain. Wind fallen. |
| 5.15 | 48 | 44 | 2.7 | 2.2 | 6.2 | 5.1 | Ditto. |
| 6.15 | 47 | 42 | 3.2 | 2.6 | 3.9 | .. | Ditto. No wind. |
| 7.15 | .. | 41 | 3.2 | 1.9 | .. | 4.4 | Ditto. |
| 8.15 | .. | 40 | 3.0 | 1.7 | 3.3 | 2.9 | Ditto. |
| 9.15 | 43 | 40 | 3.3 | 2.4 | 2.5 | 4.1 | Ditto. |
| 10.15 | 43 | 39 | 3.3 | 1.7 | .. | 1.9 | Rain stopped. Wind rising. |
| 11.15 | .. | 40 | 3.2 | 1.7 | 3.9 | 2.2 | Ditto. Wind higher. |
| 12.15 A.M. | 42 | 39 | .. | 1.7 | 6.8 | too small | Drizzling rain. Wind less. |
| 1.15 | .. | 39 | .. | 1.7 | .. | " | Fine, overcast, little wind. |
| 2.15 | 41 | 38 | 3.6 | 1.7 | 5.0 | " | Fine, and nearly clear. Wind slight. W. |
| 3.15 | .. | 38 | 4.1 | 1.7 | 6.4 | " | Ditto. |
| 4.15 | .. | 37 | .. | 1.7 | .. | 8.1 | Ditto. |
| 5.15 | 38 | 36½ | 4.1 | 1.7 | 6.8 | 4.4 | { Ditto. Mill fires lighting up in the town, and a good deal of smoke about. |
| 6.15 | 37 | 36 | 4.1 | 1.7 | 8.1 | 6.6 | |

night, when the carbonic acid is generally said to be lowest. Dundee is also close to the open sea, and without any large town near it. FODOR ('Hygienische Untersuchungen,' 1881), from a large number of observations, gives the limits as 2 to 6.

volumes, outside which cases very seldom occur. He states that carbonic acid is lowest in winter and on the seashore. LEVY ('Annuaire de Montsouris,' 1882) gives the mean carbonic acid at the observatory of Montsouris (suburbs of Paris) as 3.0 volumes. The mean carbonic acid in air appears to be distinctly lower than the quantity (4 volumes) maintained by the older authorities.

MIQUEL finds that the number of micro-organisms is also much greater in the centre of a town than in the suburbs. This is doubtless due to the stir in the town raising dust, &c (see below). Our own observation that the number of organisms is greater during the day in a town is what might be expected for the same reasons. In this connexion it will be of interest to give the results of four observations made on two different nights in one of the central courts of the Houses of Parliament in April and May of the present year.

| Time. | | Carbonic Acid. | Organic matter. | Total micro-organisms. | Bacteria. | Moulds. |
|---------------|--------------|----------------|-----------------|------------------------|-----------|---------|
| April 19-20th | 8.30 P.M. . | 4.1 | 3.1 | 9.2 | 9.2 | 0 |
| | 1.0 A.M. . | .. | .. | 4.0 | 3.6 | 0.4 |
| May 18-19th | 6.0 P.M. . | 4.1 | 2.5 | 18.0 | 14.0 | 4.0 |
| | 12.30 A.M. . | 4.4 | too small | 3.0 | 1.5 | 1.5 |

At the time of the first observation in each pair the streets were very busy.

These results, so far as they go, are in perfect accord with the conclusions deduced from the results in Dundee.

THE AIR OF DWELLING-HOUSES.

Mode and Time of taking Samples of Air.—The samples were taken during the night, between 12.30 A.M. and 4.30 A.M. This appeared to be the most favourable time for avoiding disturbing conditions, and at the same time obtaining fair specimens of the air breathed during the night. The one-roomed houses were mostly those of the very poor. Sometimes as many as six, or even eight, persons occupied the one bed. In other cases there was no bed at all. The occupants of the two-roomed houses were as a rule much better off, belonging mostly to the artisan class.

Mr. KINNEAR, the head of the Sanitary Department of Dundee, was good enough to place at our disposal a horse and covered van, by which means we were enabled to make the analyses in the street outside the houses selected for examination. Two of the inspectors belonging to the same department assisted us in our work in the case of the poorer class of houses. Those houses were visited without warning of any kind to the inhabitants, so as to avoid the risk of having the rooms specially ventilated in preparation for our visit. In every case but one we were most civilly

received, and willingly allowed to collect the necessary samples of air, measure the room, and obtain such information as was required. We were, in fact, agreeably surprised to find that so little objection was made to our untimely visit. The precaution was, of course, taken of having the door open for as short a time as possible, and of avoiding unnecessary movements such as were likely to stir up dust and contaminate the air. In the case of one-roomed houses the samples were taken about the centre of the room. In two-roomed houses we found that the door was always kept open between the two rooms, and that usually there were people sleeping in both rooms. We therefore in those cases took the samples by the door communicating between the two rooms. About ten minutes in each room were, as a rule, required in order to take the specimens, measure the room, &c., and about twenty minutes subsequently for determining the carbonic acid and organic matter in the van. Half-an-hour in all was, therefore, required for each house. We could thus visit five or six houses each night, besides taking and analysing samples of outside air in the courts or lanes in which the houses were situated.

In the case of houses of four rooms and upwards, houses of various sizes and in different parts of the town were selected, and were usually those of acquaintances. With one exception, we always found the windows and doors closed in the houses we visited. The rooms were examined in their usual conditions in every respect.

| | One roomed houses. | | | | Two-roomed houses. | | | | Houses of four rooms and upwards. | | | |
|--|--------------------|---------|----------|----------|--------------------|---------|----------|----------|-----------------------------------|---------|----------|----------|
| | No. of cases. | Lowest. | Highest. | Average. | No. of cases. | Lowest. | Highest. | Average. | No. of cases. | Lowest. | Highest. | Average. |
| Persons per house (per room in last class) | 29 | 2 | 10 | 6.6 | 13 | 4 | 10 | 6.8 | 18 | 1 | 8 | 1.3 |
| Space per person | 29 | 104 | 528 | 212 | 13 | 148 | 395 | 249 | 18 | 391 | 4206 | 1883 |
| Temperature (° F.) | 21 | 48 | 61 | 55 | 9 | 50 | 59 | 58.5 | 13 | 42 | 68 | 54.5 |
| Carbonic acid. . | 29 | 6.8 | 32.1 | 11.2 | 12 | 7.1 | 18.2 | 9.9 | 18 | 4.5 | 11.7 | 7.7 |
| Organic matter . | 29 | 7.8 | 38.1 | 15.7 | 11 | 5.0 | 30.2 | 10.1 | 18 | 1.1 | 12.0 | 4.5 |
| Total micro-organisms:— | 28 | 6.0 | 240.0 | 60.0 | 13 | 8.0 | 128.0 | 46.0 | 18 | 0.5 | 22.0 | 9.0 |
| Bacteria . . . | 19 | 6.0 | 120.0 | 58.0 | 11 | 6.0 | 118.0 | 43.0 | 16 | 0.5 | 16.0 | 8.5 |
| Moulds . . . | 19 | 0 | 5.0 | 1.2 | 11 | 0 | 10.0 | 2.2 | 16 | 0 | 1.0 | 0.4 |

The results shown in the above Table come out even more strikingly if, instead of giving the average total quantity of carbonic acid and organic matter, we give the quantities present in excess of the outside air at the time. This is done in the following Table. As the number of micro-organisms in the outside air was so small, they may be neglected, and therefore no change as regards micro-organisms is needed.

| Above outside air. | One-roomed houses. | | | | Two roomed houses. | | | | Houses of four rooms and upwards. | | | |
|----------------------------|--------------------|---------|----------|----------|--------------------|---------|----------|----------|-----------------------------------|---------|----------|----------|
| | No. of cases. | Lowest. | Highest. | Average. | No. of cases. | Lowest. | Highest. | Average. | No. of cases. | Lowest. | Highest. | Average. |
| Temperature (°F.) | 21 | 9 | 30 | 19 | 9 | 16 | 28 | 18 | 18 | 5 | 19 | 14 |
| Carbonic acid .. | 25 | 1.7 | 16.5 | 6.6 | 11 | 2.8 | 9.0 | 5.5 | 10 | 1.7 | 6.1 | 3.3 |
| Organic matter .. | 25 | 0 | 26.3 | 6.2 | 10 | 0 | 3.9 | 2.2 | 16 | 0 | 3.2 | 1.4 |
| Total micro-organisms — .. | 28 | 6 | 240 | 60 | 13 | 8 | 123 | 46 | 18 | 0.5 | 22 | 9.0 |
| Bacteria .. | 19 | 6 | 120 | 58 | 11 | 0 | 118 | 48 | 16 | 0.5 | 16 | 8.5 |
| Moulds .. | 19 | 0 | 5 | 1.2 | 11 | 0 | 10 | 2.2 | 16 | 0 | 1 | 0.4 |

Or, taking the average quantity (in excess of outside air) of carbonic acid, organic matter, and micro-organisms respectively in houses of four and more rooms as unity, then in one- and two-roomed houses we have as follows :—

| | Houses of four rooms and upwards. | Two-roomed houses. | One-roomed houses. |
|-----------------------------------|-----------------------------------|--------------------|--------------------|
| Cubic space per person | 1 | 0.13 | 0.11 |
| Carbonic acid | 1 | 1.5 | 2.0 |
| Organic matter | 1 | 1.6 | 4.4 |
| Micro-organisms (total) | 1 | 5.1 | 6.7 |
| Bacteria | 1 | 5.1 | 6.9 |
| Moulds | 1 | 5.5 | 3.0 |

The influence of cubic space per person in sleeping-rooms is indicated in the following Table, which includes all classes of houses. These are divided into seven groups, according to the cubic sleeping-space per person.

| Cubic space per person. | No. of houses. | Temperature. | Carbonic Acid. | Organic matter. | Total micro-organisms. | Bacteria. | Moulds. |
|-------------------------|----------------|--------------|----------------|-----------------|------------------------|-----------|---------|
| Cubic feet. | | | | | | | |
| 100- 180 | 14 | 55 | 11.5 | 15.1 | 80 | 78 | 18 |
| 180- 260 | 18 | 54 | 10.7 | 15.1 | 49 | 47 | 1.5 |
| 260- 340 | 6 | 53 | 10.3 | 11.8 | 32 | 31 | 0.7 |
| 340- 500 | 4 | 57 | 9.2 | 8.4 | 42 | 40 | 2.1 |
| 500-1000 | 6 | 54 | 8.6 | 5.6 | 6 | 6 | 0 |
| 1000-2500 | 8 | 53 | 6.7 | 3.9 | 9.1 | 8.5 | 0.7 |
| 2500-4000 | 4 | 57 | 7.9 | 5.0 | 13.1 | 12.8 | 0.4 |

It will be seen from the above that the carbonic acid, organic matter, and micro-organisms* all diminish in quantity as the cubic space per person increases from 100 to about 1,000 cubic feet. When, however, the cubic space increases beyond 1,000 cubic feet, the carbonic acid, organic matter, and micro-organisms show a slight, but

* See p. 95.

distinct, increase. This, unless it be a mere coincidence, appears to be an anomalous result; but it may perhaps have the following simple explanation:—A large bed-room of, say, 3,000 cubic feet has usually about the same means of ventilation as one of only 1,000 cubic feet. Consequently the air will be changed less frequently in the larger than in the smaller room, so that in the former portions, at least, of the air will be comparatively stagnant. Air vitiated by respiration will, therefore, in these portions at any rate, be removed more slowly than in a smaller room. If this be true, the results in the above Table would seem to indicate that about 1,000 cubic feet is the most appropriate sleeping-space per person in an ordinary bed-room without special means of ventilation.

In the above Table the absolute quantities of carbonic acid and organic matter are given, but similar results are obtained if we make allowance for the carbonic acid and organic matter in the outside air at the time. This has been done in the following Table:—

| Above outside air. | No. of houses. | Temperature. | Carbonic Acid. | Organic matter. | Total micro-organisms. | Bacteria. | Moulds. |
|--------------------|----------------|--------------|----------------|-----------------|------------------------|-----------|---------|
| Cubic feet. | | | | | | | |
| 100- 180 | 14 | 19 | 6.1 | 5.6 | 80 | 78 | 1.8 |
| 180- 260 | 18 | 19 | 6.9 | 6.5 | 49 | 47 | 1.5 |
| 260- 340 | 6 | 19 | 5.8 | 1.9 | 32 | 31 | 0.7 |
| 340- 500 | 4 | 18 | 5.2 | 1.9 | 42 | 40 | 2.1 |
| 500-1000 | 6 | 13 | 4.2 | 2.9 | 6 | 6 | 0 |
| 1000-2500 | 8 | 13 | 2.6 | 1.5 | 9.1 | 8.5 | 0.6 |
| 2500-4000 | 4 | 16 | 2.8 | 3.3 | 13.1 | 12.8 | 0.4 |

Comparison of Mortality Statistics with the Composition of the Air of Dwelling-Houses.

One of us, being the Medical Officer for Dundee, made arrangements for the year 1884 with the various Registrars of Deaths in the town, whereby, in addition to the information which is usually given on the registration of death, full particulars were also obtained of the number of rooms and persons in the house in which the death occurred, as well as of other similar data. This has enabled us to make a detailed comparison of the death-rates with the composition of the air in various classes of dwelling-houses.

This, we believe, is the first time such a comparison has been made, and is, in fact, the first time it has been possible, owing to the lack of necessary data. The following Table represents the results we have thus obtained. In constructing this Table, there has been a difficulty as regards the deaths occurring in the Infirmary and Poor-houses. These deaths, in the case of some diseases, materially affect the results, and, as they are almost always those of people belonging to one- and two-roomed houses, we have added a final column to the Table, in which the data for the Infirmary and Poor-

houses are added to those for the one- and two-roomed houses together. The numbers in this column for those diseases which are not materially affected by the Infirmary and Poor-houses are placed in square brackets.

The Table is divided into five sections, of which the first gives the chemical and physical data referring to the different classes of houses ; the second, the statistics of the death-rate ; the third, statistics as to the mean age at death ; and the fourth and fifth, the death-rates caused by different diseases. Those given in Section 5 are placed separately, because the number of deaths to which they refer is too small to allow of a general conclusion being drawn as to any possible connexion with the different classes of houses. The figures are, however, of sufficient interest in themselves to deserve a place in the Table.

Again, the diseases in the fourth section, and which cause more than 50 deaths, are divided into three classes, of which (A) contains those which show a complete parallelism with the number of rooms in the house ; and (B) those which show a complete parallelism only when the Infirmary and Poor-house deaths are taken into account ; while (C) includes those which do not exhibit any *evident* connexion with the class of house. The cubic spaces per person given in the first section are the means of our own observations, though it is probable that they are somewhat lower than they would be for the whole town. Unfortunately we have no statistics as regards the condition of the air in 3-roomed houses.

A consideration of the Table shows :—

(1.) That, as we pass from 4-roomed and upwards to 3-, 2-, and 1-roomed houses, not only does the air become more and more impure, as indicated by the increase in the carbonic acid and organic matter, and more especially of the micro-organisms, but that there is a corresponding and similar increase in the death-rate, together with a marked lowering of the mean age at death.

(2.) That the rapid increase in the death-rate as we pass from 4- to 1-roomed houses is by far the most marked in children under five ; that the death-rate among these young children in 1-roomed houses is nearly four times as great as in 4-roomed houses, whereas the general death-rate is not quite twice as great ; further, that although there is still a marked increase in the death-rate for all above five years of age in the smaller houses, yet this increase is comparatively small, and is not evident unless the deaths in the Infirmary and Poor-houses be included in the 1- and 2-roomed houses. The death-rates of persons above 70 and also above 80 years of age in the different classes of houses is likewise interesting and instructive. In each of these cases the death-rate rapidly increases from 1- to 4-roomed houses ; showing, not that persons above these ages are more likely to die in 4-roomed than in 1-roomed houses, but that there are more persons of advanced age living in the better class than in the 1-roomed houses.

(3.) The mean age at death in the better-class houses is almost twice as great as in 1-roomed houses. Persons living in 1-roomed houses have, therefore, the chance at

birth of living only one-half as long as those in better-class houses, or they die nearly 20 years sooner, on the average, than those of the better class. This is an enormous difference. If we take the mean age of those who died above 20, we find that a similar lowering of the mean age at death likewise occurs in the worse class of houses, though not nearly to such a marked degree. It is, in fact, only the strong ones who have survived in the 1- and 2-roomed houses, the weaker ones having been mostly cut off before they reach the age of 5 years. The higher mean age at death of those who died above 20 years of age in 1-roomed houses, though possibly due partly to the fact that a larger proportion of the people living in 1-roomed houses are employed in outside labour during the day than is the case with the other classes, is, doubtless, chiefly due to a process of natural selection, whereby the weaker ones have been taken off earlier in life, so that those who are left are much more able to combat circumstances unfavourable to life than are those in 2- and 3-roomed houses, and who have not undergone this natural selection to nearly such a great extent.* The Table also shows that in better-class houses persons above 70 are likely to live about a year longer than those above 70 years of age in 1- and 2-roomed houses, although the latter are to a much greater extent a "survival of the fittest."

(4.) As regards deaths from particular causes, those from phthisis require special notice.

As is well known, and as other statistics† have conclusively proved, the prevalence of phthisis is very largely affected by the state of purity of the air. It might have been expected, therefore, that the death-rate from phthisis especially would show a marked parallelism with differences in the condition of the air, and, consequently, in the

* KÖRÖSI (see 'Annales d'Hygiène Publique,' vol. 14, 1885, p. 571) finds that for the years 1874-1881 in Buda-Pesth the mean age at death was :—

| | Of all who died | |
|--------------------------|-----------------------|-----------------------|
| | Below 5 years of age. | Above 5 years of age. |
| Among the rich | 1·3 years | 52·0 years |
| „ middle class | 1·2 „ | 46·1 „ |
| „ poor | 1·0 „ | 41·6 „ |

Or, arranged according to the class of houses, as follows :—

| Of all who died above 5 years of age. | |
|---------------------------------------|------------|
| Best class of houses | 44·2 years |
| Middle „ „ | 42·2 „ |
| Worst „ „ (cellars) | 39·9 „ |

† See PARKES' 'Hygiene,' 6th edition, page 133 *et seq.*, also page 105, in which a large number of facts are adduced, of which the following may serve as examples :—

Two Austrian prisons, in which the diet and mode of life were, it is believed, essentially the same, offer the following contrast :—In the prison of Leopoldstadt, at Vienna, which was very badly ventilated, the death-rate in 1834-1847 was 86 per 1,000, and of these no less than 51·4 died from phthisis.

In the well-ventilated House of Correction in the same city, the death-rate (1850-1854) was only 14 per 1,000, and of these 7·9 died of phthisis.

class of house. This, however, is not so according to the Table, for the death-rate from phthisis is much the highest in 3-roomed houses, and then diminishes to 1-roomed houses, in which it becomes almost as low as in better-class houses. When the deaths from phthisis in the Poor-houses and Infirmary are set down against those living in 1- and 2-roomed houses, this class has still a lower death-rate from phthisis than those living in 3-roomed houses. On consideration, the explanation of this appears to be quite simple. Deaths from phthisis do not usually occur much under 20 years of age, tubercular disease under that age usually taking other forms. Now those living in 1-roomed houses, and who would be most liable to be attacked by phthisis, have been already killed off at a much earlier age by diarrhoea, acute bronchitis, broncho-pneumonia, tubercular meningitis, etc.; hence the smaller death-rate from phthisis in 1- and 2-roomed houses. In fact, the diseases just mentioned may be almost considered as the complement of phthisis, so that as the one increases the total of the others diminishes, but less rapidly: their sum, therefore, still shows a marked increase from 4- to 1-roomed houses.

| | Number of rooms. | | | |
|--|------------------|------|------|------|
| | 4 and upwards. | 3 | 2 | 1 |
| Death-rate per 10,000 from :— | | | | |
| Diarrhoea, acute bronchitis, broncho- pneumonia, and meningitis . . } | 19·6 | 27·6 | 39·7 | 59·8 |
| Phthisis } | 13·0 | 27·6 | 24·4 | 14·6 |
| Total from all the above causes . | 32·6 | 55·2 | 64·1 | 74·4 |

Were only the cases of diarrhoea occurring during childhood included in the first category, the above figures would become still more striking.

(5.) The considerable increase in acute bronchitis, broncho-pneumonia, etc., as we pass from 4- to 1-roomed houses, fully confirms, though in a different way, previous observations as to the effect of impure air in promoting pulmonary diseases. A very conclusive example of this is given in the report by Deputy Surg.-General SIMPSON (and quoted in PARKES' 'Hygiene,' 6th edit., p. 135) on the health of the South Afghanistan field-force during the time they wintered at Candahar in 1880-81.

(6.) Of those diseases which are usually considered infectious, measles, whooping-cough, and diphtheria (including croup) are the only ones for which there are sufficient deaths to allow a conclusion to be drawn. Of these, the mortality from measles and whooping-cough, but especially the former, shows a very distinct connexion with the

class of house. Contrary to expectation, the mortality from diphtheria and croup does not show any connexion with the class of house.*

(7.) It is seen that deaths certified as being due to "old age" do not run parallel with the different classes of houses, though one would have expected that they would have done so, and that the numbers would have diminished from 4- to 1-roomed houses. "Old age" is a somewhat indefinite term as a cause of death, and would be materially affected by the mode in which the doctors filled in the certificates; so that it does not form a safe indication of the number of people of advanced age living in the various classes of houses. A reliable indication of this, however, is furnished by the death-rate of persons who die above 70 or above 80 years in the different classes of houses; and, as already remarked above, this shows a complete parallelism, and proves conclusively that there is a much larger proportion of old people living in the better than in the worse class of houses.

The effects of impurity in air on the death-rates from different diseases are more particularly discussed below (page 105).

THE AIR OF SCHOOLS.†

In the course of our investigations we have examined the following schools and other educational institutions in Dundee:—

(a.) *Sixteen ordinary Board schools* (all the schools under the Dundee Board).—Two rooms were examined in each school. Some of the schools were heated by fires and others by hot pipes. They were all ventilated by the ordinary means usually adopted in such buildings, viz., fires, ventilators in the roof, and open windows. The great majority of the latter opened by means of hinged panes, opening obliquely so as to direct the entering current upwards.

(b.) *The Harris Academy*.—This school is also under the Board, but is of a higher grade than the ordinary Board schools, and the children are of a higher class. Fifteen rooms were examined in this school on various occasions and under different conditions. Of those examined, twelve rooms were mechanically and three naturally ventilated.

(c.) *A half-time school* belonging to one of the large mills in the town.—The two rooms (one for girls and the other for boys) were frequently examined under different conditions. The children in this school are employed half the day in the mill, and are at school the other half. This school was ventilated mechanically.

(d.) *Two denominational schools*.—Two rooms in each; these were naturally ventilated and heated by fires.

* KÖRÖSI (*loc. cit.*) has shown that the mortality from infectious diseases generally increases with the density of the population, and that this is most marked in the case of hooping-cough and measles, but is not evident with scarlet fever and diphtheria. (Of. below, p. 107.)

† See also Supplementary Note. p. 111.

(e.) *One private school (girls).*—Three rooms examined. Heated by fires and ventilated naturally.

(f.) *The Dundee High School.*—Six rooms examined. Ventilated mechanically.

(g.) *The two Lecture-rooms and large general laboratory of the Chemical Department, University College.*—These were frequently examined under various conditions. Ventilated mechanically.

We have thus examined no less than sixty-eight different schools and college class-rooms, and some of these many times, under different conditions as regards ventilation.

Of these forty-two were ventilated in the ordinary way by fireplaces, windows, &c. (natural ventilation), twenty-six were ventilated by fans which blew air into the rooms (mechanical ventilation).

The comparatively large number of schools in Dundee which are ventilated mechanically make it a very good field for testing the relative efficiency of natural and mechanical ventilation. The large number of data we have thus been able to obtain in schools ventilated on the two systems will, we think, be of considerable interest, not only to educationalists, but to all who have to do with the ventilation of public buildings.

The method adopted in all those rooms which are mechanically ventilated* is to blow air by fans over hot pipes, and thence into the several rooms by broad, shallow, upright shafts, opening at a height of five feet from the floor. The vitiated air is taken off by shafts which open two feet from the floor and carry the air up into a chamber in the roof. Thence it is discharged through louvre-boarded ventilators, fitted inside with valves, which prevent any possibility of back draughts. As a rule there is an outlet shaft at each end of the room, and one or more inlet shafts on each side. The air on entering the room thus passes wholly or partially towards the ceiling, and must thence pass downwards to find an exit by the upcast shafts, which open near the floor. The current is intended to sweep the whole room in this way, while the broad and shallow inlet shafts, through which a large volume of air enters at a low velocity, ensure a good distribution of air with as little draught as possible.

All the schools were examined during the winter months, between December 16, 1885, and April 28, 1886. They were visited without previous warning, except in one or two cases where special experiments were to be made, such as having the ventilating fans stopped, &c. We observed the state of the windows before entering; and the masters were always good enough to keep them in the same condition during our observations as that in which we found them. Hence, if any windows were open on our arrival they remained so, or if shut they remained shut, our object being to have everything under the usual conditions so far as possible. The samples were collected as near the centre of the room as possible. The results are stated in the following Table :—

* For all these Mr. Wm. CUNNINGHAM, of Dundee, was the engineer.

| | Schools. | | | | | | | |
|--|-----------------------|---------|----------|---------|--------------------------|---------|---------|---------------|
| | Naturally ventilated. | | | | Mechanically ventilated. | | | |
| | No of cases. | Lowest. | Highest. | Average | Average. | Lowest. | Highest | No. of cases. |
| Per cent of windows open | .. | .. | .. | 22 | 3 | .. | .. | .. |
| No. present, including staff | 39 | 27 | 191 | 92 | 64 | 20 | 170 | 20 |
| Space per person | 39 | 58 | 427 | 168 | 164 | 119 | 228 | 20 |
| Temperature (° Fahr.) | 35 | 44 | 65 | 55.6 | 62 | 53 | 69 | 18 |
| Carbonic acid | 39 | 7.9 | 37.8 | 18.6 | 12.3 | 7.0 | 19.6 | 20 |
| Organic matter | 38 | 5.0 | 40.3 | 16.2 | 10.1 | 3.4 | 19.0 | 20 |
| Total micro-organisms — | 35 | 8 | 600 | 152* | 16.58* | 0 | 53 | 18 |
| Bacteria | 23 | 8 | 600 | 151 | 16.0 | 0 | 53 | 18 |
| Moulds | 28 | 0 | 4 | 1.1 | 0.58 | 0 | 2 | 13 |
| Or above outside air :— | | | | | | | | |
| Temperature (° Fahr.) | 25 | 3 | 34 | 16.8 | 24 | 22 | 26 | 3 |
| Carbonic acid | 39 | 4.4 | 34.3 | 15.1 | 8.9 | 3.5 | 16.1 | 20 |
| Organic matter | 38 | 0 | 31.4 | 7.8 | 1.1 | 0 | 5.3 | 20 |

Or, if we take as units the average cubic space, the average excess over outside air of temperature, of carbonic acid, and of organic matter, and the average micro-organisms, in mechanically ventilated schools, the comparative results for naturally ventilated schools may be expressed as in the following Table :—

| | Mechanically ventilated. | Naturally ventilated. |
|--|--------------------------|-----------------------|
| Cubic space per person | 1 | 1.0 |
| Temperature in excess of outside air | 1 | 0.66 |
| Carbonic acid " " | 1 | 1.7 |
| Organic matter " " | 1 | 7.0 |
| Micro-organisms " " | 1 | 9.2 |
| Bacteria " " | 1 | 9.4 |
| Moulds " " | 1 | 2.0 |

The above Table shows that with mechanical ventilation, the space per person being the same :—(1) The carbonic acid was three-fifths, the organic matter one-seventh, and the micro-organisms (see pp. 96, 97–98) less than one-ninth of what they were in schools ventilated by ordinary methods. (2) That, notwithstanding this very great improvement in the purity of the air, the temperature is very considerably higher in the mechanically ventilated schools.

To produce such improvement in purity by the ordinary methods of opening windows, &c., would have reduced the temperature to a very uncomfortable and dangerous degree. The improvement is also obtained with comparatively little*

* The marked difference between these two classes of schools is shown still more distinctly by the fact that of the mechanically ventilated schools only two contained more than 26 micro-organisms per litre, whereas of the naturally ventilated schools only three contained less than 26 per litre.

perceptible draught. When a draught is perceptible it is a warm, and not a cold draught, as is the case with ventilation by an open window.

Mechanical ventilation does not merely reduce the number of micro-organisms during the time it is in action, but has, as will be shown below (p. 97), a marked effect after it has been stopped and replaced by natural ventilation, this effect extending over a period of many days at least.

Further, mechanical ventilation, as shown by Professors BRAZIER and NIVEN, of Aberdeen University (see below), keeps the composition of the air more or less constant at different points in a room, whereas with natural ventilation it is liable to be much more impure at one part than another.

We have not included in the above Table the Dundee High School nor the only private school we have examined, as in these two cases the cubic space per person was about three times as great as in the other schools. The results for these two schools were as follows. It will be seen that practically they confirm the conclusions drawn from the results in other schools, though the effects of mechanical ventilation are not nearly so marked. The reasons for this will appear subsequently (page 98).

| | Private school. (Girls.) Naturally ventilated. | | | | Dundee High School. (Boys and girls.) Mechanically ventilated. | | | |
|---------------------------|--|---------|----------|----------|--|---------|----------|------------------------------|
| | No. of rooms examined. | Lowest. | Highest. | Average. | Average. | Lowest. | Highest. | No. of rooms examined. |
| Number present | 3 | 6 | 11 | 9 | 36 | 13 | 64 | 6 |
| Space per person | 3 | 320 | 528 | 457 | 588 | 320 | 1102 | 6 |
| Temperature (° Fahr.) . . | 3 | 56 | 57 | 57 | 57 | 51·5 | 60·5 | 6 |
| Carbonic acid | 3 | 10·7 | 13·3 | 11·9 | 13·0 | 8·5 | 18·4 | 6 |
| Organic matter | 3 | 6·2 | 11·8 | 8·9 | 3·9* | 1·7 | 5·6 | 6 |
| Total micro-organisms . . | 3 | 4 | 15 | 9·3 | 3·6 | 1 | 11 | 7 |
| Bacteria | 3 | 4 | 15 | 9·0 | 2·9 | 1 | 10 | 7 |
| Moulds | 3 | 0 | 1 | 0·3 | 0·7 | 0 | 3 | 7 |

Last year Professors BRAZIER and NIVEN made a report to the Aberdeen School Board on the ventilation of schools in that town. From this report it appears that they examined (the carbonic acid only being determined) four different schools ventilated in the ordinary manner, and two schools ventilated mechanically by fans. From their detailed results we have calculated that the average temperature and carbonic acid in excess of the outside air were as follows :—

*A determination of the organic matter in the outside air was not made when the High School was examined; but, as the outside organic matter on the day we visited the private school amounted to only 1·6, the private school must have been considerably in excess of the High School, even allowing nothing for the outside air when the latter school was examined.

| | Temperature. (Fahr.) | Carbonic acid. | | Ratio. | |
|-----------------------------|-------------------------|----------------|----|--------------|----------------|
| | | | | Temperature. | Carbonic acid. |
| Mechanically ventilated . . | 16 | 11.0 | Or | 1.0 | 1.0 |
| Naturally ventilated . . . | 14.6 | 17.0 | | 0.9 | 1.6 |

It will be seen that, though the average excess of carbonic acid found by them was somewhat higher than what we have found from the examination of a much larger number of schools, yet the ratio is practically the same (1 to 1.6 instead of 1 to 1.7). Like us, they found the excess of temperature over outside air greater in the mechanically than in the naturally ventilated schools, though the difference is not so marked in their observations as in ours. They conclude that mechanically ventilated schools "compare very favourably with those on the other system." We, however, would speak much more strongly than this in favour of mechanical ventilation, since it not only considerably reduces the carbonic acid, but effects a very much greater reduction of those impurities which are undoubtedly far more injurious to health than the usual excess of carbonic acid. It does this also without producing the very objectionable fall in temperature necessarily associated with effective natural ventilation. We entirely agree with Professors BRAZIER and NIVEN in believing that the system of ventilating by open windows is, for winter, at least, very objectionable. The severe draughts thus produced are possibly a worse evil than defective ventilation.

It is true that mechanical ventilation is apparently more costly; but it remains to be proved that it really is so. As our results show, the cubic space per child would not require to be nearly so great, in order to maintain a given standard of purity, on the mechanical as on the "natural" system. Hence the cost of building would be less. The cost of heating would also be reduced, on account of the smaller space to be heated. The reduction in space could most advantageously be made in the height of the rooms.

The chief difficulty in connexion with mechanical ventilation is to maintain a proper distribution of the air, and consequently of the heat, in several rooms within the same building. With proper care, however, there is no reason why this should not be accomplished.

The all-important argument for mechanical ventilation is that it maintains a certain standard of purity, and, unless some simpler method which will maintain a similar standard can be devised, its adoption in crowded schools seems to be very much required.

When we come to consider that the children who attend average Board schools for six hours a day are during that time subjected to an atmosphere containing on an average nearly 19 volumes of carbonic acid per 10,000, and a very large proportion of organic matter, and no less than 155 micro-organisms at least per litre, we

need not be surprised at the unhealthy appearance of very many of these children. It must also be borne in mind that many of them are exposed for nine hours more to an atmosphere which, as we have shown above, is about five times as impure as that of an ordinary bed-room in a middle-class house. They are thus breathing for at least fifteen hours out of the twenty-four a highly impure atmosphere. The effects of this are often intensified, as is well known, by insufficient food and clothing, both of which must render them less capable of resisting the impure air. The fact that these schools become, as will be shown below, after a time habitually infected by bacteria renders it probable that they also become permanent foci of infection for various diseases, and particularly perhaps for tubercular disease in its various forms. From the considerations advanced later on (page 106), it will be seen how an ordinary simple cold, brought on say by a draught, may become a source of great danger to a child attending such a school.

The *cubic space per person in schools*, unlike that in houses, shows no definite connexion (as will be seen below) with the purity of the air, except as regards the number of micro-organisms. In mechanically ventilated schools these diminish in a marked manner with increase of cubic space. In naturally ventilated schools, on the other hand, the number of micro-organisms was found to increase as the cubic space increases from 50 to 250 cubic feet, after which it diminished (cf. pp. 93, 94, 95).

| Cubic space per person | Naturally ventilated. | | | | Mechanically ventilated. | | | |
|------------------------|-----------------------|----------------|----------------|-----------------------|--------------------------|----------------|----------------|-----------------------|
| | No of cases | Carbonic Acid. | Organic matter | Total micro-organisms | No of cases | Carbonic Acid. | Organic matter | Total micro-organisms |
| Cubic feet | | | | | | | | |
| 50-100 | 6 | 21.5 | 16.2 | 119 | 7 | 14.0 | 7.8 | 23 |
| 100-150 | 14 | 15.5 | 19.6 | 128 | 8 | 11.4 | 9.6 | 14 |
| 150-200 | 5 | 18.9 | 12.8 | 150 | 5 | 11.8 | 12.3 | 10 |
| 200-250 | 9 | 21.1 | 16.8 | 188 | .. | .. | .. | .. |
| 250-300 | 4 | 17.1 | 9.5 | 187 | 6 | 13.0 | 3.7 | 2 |
| 300 and upwards | 4 | 15.1 | 11.8 | 12 | | | | |

Boys' and Girls' School-rooms.—The difference between boys' and girls' rooms is most marked. Out of fifteen pairs of rooms, one room of each pair being occupied by boys and the other by girls, and under circumstances* as nearly as possible the same, no less than fourteen gave a *much lower* result in favour of the girls as regards micro-organisms, whilst the only exception against the girls was a room in one of the denominational schools. This room was lighted only by two skylights, though in

* Such as class of children, mode of ventilating, cubic space, time of experiment, &c., and, with but one exception, in the same building; in this exception the two schools were in connexion with one another.

other respects similar to the boys' room, which was lighted by windows (open) and had only half as much space per person as the girls' room.

In ten pairs which could be compared for carbonic acid eight were *very largely* in favour of the girls, whilst in one of the two exceptions the girls were only *very slightly* in excess of the boys. As regards the oxidisable organic matter, there were, out of nine pairs which could be compared, six *largely* in favour of the girls, whilst of the three exceptions two were only slightly in excess of the boys. With two exceptions, out of ten pairs of rooms compared, the temperature with the girls was always lower than with the boys.

The average results are given in the following Table :—

| | Space per person. | Temperature. (° Fahr.) | Carbonic Acid. | Organic matter. | Micro-organisms | | |
|-------------------------|-------------------|------------------------|----------------|-----------------|-----------------|----------|--------|
| | | | | | Total. | Bacteria | Moulds |
| No. of rooms compared . | 30 | 20 | 20 | 16 | 30 | 30 | 30 |
| Boys | 275 | 60 | 15.0 | 7.9 | 92 | 90 | 2 |
| Girls | 382* | 58 | 12.3 | 6.7 | 65 | 64 | 1 |

It is thus seen that boys tend to make the air of a room more impure than girls do, and that consequently they require a more efficient ventilation in order to maintain a given standard of purity in the air of their rooms. The reasons for this are not far to seek, and may be stated as follows :—(1) The boys are more restless, and so raise more dust, which necessarily contains micro-organisms (see below). For the same reason they evolve more carbonic acid, and probably organic matter. (2) The girls are, as a rule, cleaner, and this has a marked effect (as will be shown below) in diminishing the number of micro-organisms. (3) Boys usually come to school after more violent exercise than girls, which results in the production of more carbonic acid, and probably of organic matter (see below). (4) From differences in concentration, more or less apart from the above reasons, boys evolve more carbonic acid, and perhaps of organic matter (see PARKES' 'Hygiene,' 6th edn., p. 114). Or, to sum up some of the above reasons, and put them from a slightly different point of view, boys eat more than girls.

THE AIR OF MILLS AND FACTORIES.

We have only examined four of these, samples having been taken from two rooms in each. As the results obtained differ very widely in the several cases, we are not

* This cubic space in favour of the girls is caused by the space per person in one of the rooms being very much larger than in any other.

able to draw any very definite conclusions in respect to them. Such results, however, as we have obtained are expressed in the following Table. All the works examined were engaged in the manufacture of jute and tow, this being the staple trade of Dundee. The visits were all made without previous intimation as to the time of our visit.

| | No of rooms examined | Lowest | Highest | Average |
|-----------------------|-------------------------|--------|---------|---------|
| No of persons present | 8 | 6 | 500 | 157 |
| Space per person | 8 | 593 | 5485 | 1773 |
| Temperature (° Fahr) | 6 | 45 | 58 | 53 |
| Carbonic acid | 8 | 4.8 | 23.2 | 13.3 |
| Organic matter. | 8 | 5.3 | 36.1 | 17.4 |
| Total micro-organisms | 6 | 4 | 600 | 160 |
| Bacteria | 6 | 4 | 586 | 114 |
| Moulds | 6 | 0 | 600 | 125 |

A room in one of the mills gave a total of (at least) 260 micro-organisms per litre, of which 12 were bacteria and 248 were moulds. The moulds in this case grew very rapidly, and finally filled and choked up the tube. They had a very beautiful and delicate appearance, like frosted glass, and with a woolly texture. Being much struck with the character of this growth, and not having obtained anything similar in any other case, we made a second examination of the room five weeks after the first visit. The result was the same as before, except that the moulds grew even more rapidly and luxuriantly. This time they amounted to at least 586 per litre. The predominating species of mould appeared to be exactly the same as in the first case, and it rapidly overgrew any other moulds or bacteria which appeared in the tube. One of the tubes containing these moulds is represented in Plate 6 (fig. 1).

What the cause was of the prevalence of this mould in the room we could not discover at the time. We subsequently learned, however, that about eight months previous to our visit the owners of the mill had purchased, and subsequently manufactured, a considerable quantity of loose re-dried jute which had been saved from a stranded vessel. This may possibly have been the original source of the moulds which had come to prevail in the mill.

DUNDEE ROYAL INFIRMARY.

We also made an examination of four different wards in the Infirmary. One series of observations was made in the afternoon between 4.30 P.M. and 5.5 P.M., and the other in the early morning of the following day between 2.40 A.M. and 5.30 A.M. Previous intimation had, of course, to be given in this case. The wards are heated by hot pipes and fires, and ventilated on the natural system. All the windows (about ten) in each of the wards were open about one inch at the time of our visit. The Infirmary

is situated in a large open space on a hill at the back and on the north side of the town. The results were as follows:—

| March 26th and 27th, 1886. | | Time. | Gas jets burning. | Temperature. | No of persons, including nurses | Space per person | Carbonic acid. | Organic matter. | Total micro-organisms. | Bacteria. | Moulds. | Remarks. |
|-----------------------------------|------|-------|-------------------|--------------|---------------------------------|------------------|----------------|-----------------|------------------------|-----------|---------|---|
| | | | | ° Fahr. | | | | | | | | |
| Accident Ward | p m. | 4.30 | 0 | 59 | 18 | 1368 | 5.3 | 8.9 | 2.5 | 2 | 0.5 | Felt very fresh |
| Children's Ward | | 4.40 | 0 | 59 | 29 | 1084 | 6.1 | 6.3 | 2 | 2 | 0 | Not close, but a distinct odour very perceptible. |
| Medical Ward No. 12 | | 5.0 | 0 | 61 | 24 | 1458 | 4.9 | 7.5 | 5 | 1 | 4 | Somewhat faint close smell. |
| " No 10 | | 5.5 | 0 | 62 | 11 | 3163 | 4.3 | 6.6 | 0 | 0 | 0 | Felt fresh; scarcely any smell. |
| Outside air in front of Infirmary | | 6.20 | --- | 43 | --- | --- | 3.2 | 3.3 | .. | --- | .. | Wet rainy day. Windy. S E. |
| | | | | | | | | | | | | |
| Children's Ward | a m. | 2.40 | 2 | 59 | 28 | 1071 | 6.9 | 1.9 | 6 | 2 | 4 | Scarcely any smell; much fresher than in day. |
| Medical Ward No. 12 | | 3.25 | 2 | 60 | 23 | 1522 | 5.5 | 2.0 | 4 | 1 | 3 | Scarcely any smell. |
| Outside air as before | | 5.0 | --- | 43 | --- | --- | 3.5 | 3.6* | --- | --- | --- | Fine, but somewhat overcast. Mill chimneys, tops of which were on level with Infirmary, were turning out black smoke in large quantity. |
| Accident Ward | | 5.10 | 1 | 53 | 16 | 1388 | 7.6 | 5.1 | 23 | 26 | 2 | Fresh; scarcely any smell. |
| Medical Ward No. 10 | | 5.25 | 1 | 60 | 11 | 3162 | 4.1 | 5.7 | 0 | 0 | 0 | No smell; fresh. |

If the ventilation was in its normal condition, the above results indicate a very satisfactory state of things as regards the air of the Infirmary wards.

Having now described the results obtained in the various classes of buildings, the air of which was examined, we must now turn to certain special points of general interest.

Relation of Quantity of Carbonic Acid to Quantity of Organic Matter and Number of Micro-organisms.

No constant relation between the quantities of carbonic acid, organic matter, and micro-organisms can be detected in individual cases (see PARKES, p. 147; also DE CHAUMONT, 'Roy. Soc. Proc.,' vol. 23, p. 188). Sometimes we find a high organic matter accompanied by a low carbonic acid, whilst under other circumstances the reverse may be the case. A determination of carbonic acid alone is therefore never a sufficient indication of the purity or otherwise of a given sample of air. Nevertheless, by taking the average of a considerable number of observations, we find that there is a *general* relationship, so that a high carbonic acid is, as a rule, accompanied by a high organic matter, and *vice versa*, though this is by no means always the case. There appears, however, to be no definite connexion between the number of micro-organisms and the amount of carbonic acid (see page 93).

* The sudden increase in oxidisable organic matter at this time was due to the firing up of the boilers at the mills, the tops of the chimneys of which were on a level with the Infirmary, and large quantities of thick black smoke were being turned into the air.

These conclusions will be seen to follow from the accompanying Tables:—

| | Carbonic acid | Organic matter. | Total micro organisms | No of cases |
|---|---------------|-----------------|-----------------------|-------------|
| | Mean | Mean. | Mean. | |
| With 2- 4 volumes Carbonic acid* | 2.7 | 5.6 | 1.9 | 56 |
| " 4- 6 " " | 4.9 | 6.1 | 3.5 | 27 |
| " 6- 8 " " | 7.3 | 10.4 | 29.7 | 25 |
| " 8-10 " " | 8.9 | 9.5 | 33.5 | 26 |
| " 10-12 " " | 11.1 | 11.4 | 79.6 | 29 |
| " 12-15 " " | 13.3 | 11.8 | 36.3 | 27 |
| " 15-20 " " | 17.0 | 13.0 | 137.0 | 31 |
| " 20-30 " " | 22.9 | 13.6 | 82.0 | 12 |
| " 30 and above " | 37.1 | 19.8 | 53.0 | 9 |
| With 0- 2.8 volumes of Oxygen required for organic matter | 4.3 | 1.6 | 5.3 | 21 |
| " 2.8- 5.6 " " " | 6.8 | 4.2 | 10.1 | 52 |
| " 5.6- 8.4 " " " | 9.7 | 7.2 | 34.1 | 58 |
| " 8.4-11.2 " " " | 10.7 | 9.7 | 29.2 | 24 |
| " 11.2-14.0 " " " | 11.9 | 12.6 | 88.3 | 31 |
| " 14.0-16.8 " " " | 16.0 | 15.4 | 57.1 | 17 |
| " 16.8-22.4 " " " | 18.5 | 19.3 | 145.0 | 17 |
| " 22.4 and above " " " | 18.8 | 29.7 | 87.0 | 15 |

In the first, the other constituents are compared with the carbonic acid as standard; in the second, with the organic matter as standard.

CIRCUMSTANCES AFFECTING THE ORGANIC MATTER IN AIR.

The most important circumstances which suggest themselves as likely to affect the amount of oxidisable organic matter in air are the following:—(1) Combustion of coal, (2) ditto of coal gas and of oil, (3) respiration, (4) dust, (5) physical exercise, (6) cleanliness.

1. *Combustion of Coal*.—A close connexion is traced between the amount of organic matter present in air and the combustion of coal. This point has been investigated by one of us in conjunction with Mr. WM. MACKIE ('Roy Soc. Proc,' vol. 41, p. 238), and as a result of that investigation it was found that in town the organic matter was lowest during the night, rather higher in the morning (5 A.M. to 10 A.M.), considerably higher in the middle of the day (10 A.M. to 3 P.M.), and higher still towards evening (3 P.M. to 8 P.M.), after which it decreased. It is generally rather high in the early morning (5 A.M. to 7 A.M.), when fires are being lit, and the black smoke of incomplete combustion discharged from the chimneys (compare pp. 65-69; also PARKES, p. 117).

In examining the air of a room for organic matter, it is therefore necessary also to determine the condition of the outside air at the same time.

2. *The Combustion of Coal Gas* does not appreciably increase the quantity of organic

* Compare PARKES, 6th edn., p. 116.

matter in air, whereas a burning oil lamp has a very marked effect in this respect (see CARNELLEY and MACKIE, *loc. cit.*).

3. *Respiration*.—CARNELLEY and MACKIE (*loc. cit.*) have also shown that the quantity of organic matter in the air of a room becomes greater as the period of vitiation by respiration increases. In this connexion we have made a set of experiments with the object of determining the amount of organic matter in undiluted expired breath. For this purpose the observer inspired the air of the room through his nose, and expired through the mouth into a closed bottle of about $3\frac{1}{2}$ litres capacity, and provided with a small outlet tube for the escape of the excess of expired air. This bottle was maintained at a temperature of about 45° C. by immersion in warm water, in order to prevent condensation of moisture from the breath. When the bottle was full of expired air, for which 50 expirations were considered sufficient, the temperature of the enclosed air was observed, the inlet and outlet tubes closed, and the bottle removed from the bath and allowed to cool down to the temperature of the room, when the inlet tube was opened and air allowed to enter to fill the partial vacuum. The temperature of the enclosed air was again observed, and the amount of organic matter determined in the usual way. A determination of the organic matter in the air of the room was likewise made at the same time. The proportion of expired and unrespired air of the room in the bottle could be found by calculation. Then, by deducting from the total organic matter that present in the known proportion of unrespired air, the difference gave the amount of organic matter in undiluted breath. Care was taken to breathe as nearly as possible in a natural manner. The results obtained were as follows :—

| Observer A. | | | Observer B. | | |
|-----------------------|-----------------|------------------------|-----------------------|-----------------|------------------------|
| Total in expired air. | In air of room. | Excess in expired air. | Total in expired air. | In air of room. | Excess in expired air. |
| 3.3 | 1.6 | 1.7 | 6.5 | 3.0 | 3.5 |
| 12.4 | 3.2 | 9.2 | 12.2 | 1.6 | 10.6 |
| 5.8 | 1.6 | 4.2 | 13.3 | 4.7 | 8.6 |
| 11.8 | 2.2 | 9.6 | 13.1 | 1.9 | 11.2 |
| 15.6 | 2.0 | 13.6 | 10.1 | 2.3 | 7.8 |
| Average per litre | | 7.6 | Average per litre | | 8.3 |

The above determinations were mostly made on different days. According to these experiments the amount of oxidisable organic matter in breath is by no means constant, but varies from time to time, nor is the quantity so great as one might have expected. It is possible, however, that the organic matter in freshly expired breath is not in a condition to readily reduce permanganate, but after exposure for some time in the air it may undergo such a change as will render it more readily oxidisable.

4. *Effect of Dust and of the Stagnation of the Air.*—An atmosphere which has been entirely at rest for some time contains less organic matter than it did previously. This, however, is not necessarily due altogether to the settling down of solid organic dust (though dust materially affects the determinations), but is probably owing in part to oxidation. The effect of stagnation, and thence of settling, and possibly of partial oxidation, was shown by the following results :—

| | Organic matter. | Second experiment. |
|--|-----------------------|--|
| Air of dark cellar which had been kept closed for some time | 9.4 | 1.7 Too small to estimate. Ditto |
| Outside air at same time | 13.2 | |
| Air-tight room after being well ventilated with outside air and then closed for three days . | 4.9 | |
| Outside air on third day | 9.5 | |
| Same room after ventilation with outside air | 3.2 | |
| Ditto, after being closed for two days . . { | Too small to estimate | |
| Ditto, after ventilation with outside air . . | 1.7 | |
| Same room after five persons had been in the room for $\frac{1}{2}$ -hour with the door closed . . | 3.2 | |
| Ditto, after the room had been closed for two days | 1.7 | |
| Organic matter in outside air at end of period | 1.7 | |

A similar effect is also shown in the case of the large sewer under the Houses of Parliament. This sewer forms a cul-de-sac at the end by the Victoria Tower, whence it runs the whole length of the building to the Clock Tower, where it joins the Metropolitan sewer. Before the recent alterations, this sewer was ventilated by suction, caused by a furnace at the bottom of the Clock Tower. Practically, therefore, this suction was pulling against what tended to become a vacuum; the effect being that, though there was a good draught by the Clock Tower, the strength of the air-current gradually diminished as the blind end of the sewer was approached, so that near the Victoria Tower end no draught was perceptible. Here the air remained stagnant. Suspended organic matter would thus settle down, and in consequence of this and of oxidation there would be an increase in carbonic acid, accompanied by a diminution in the organic matter, as we pass from the Clock Tower end to the Victoria Tower end of the sewer. This is shown as follows :—

| | First determination. | | Second determination. | |
|--|----------------------|-----------------|-----------------------|-----------------|
| | Carbonic acid. | Organic matter. | Carbonic acid | Organic matter. |
| In the sewer by the Clock Tower end . . . | 7.3 | 12.9 | 5.2 | 11.8 |
| Ditto, midway between the Clock and Victoria Towers | 8.2 | 13.0 | 7.0 | 9.5 |
| Ditto, by the Victoria Tower | 8.5 | 9.4 | 6.5 | 9.4 |

The first determinations were made between the hours of 5 P.M. and 7.30 P.M., and the second between 10.30 P.M. and 12 midnight.

5. *Effect of Physical Exercise.*—As is well known, physical exercise causes a marked increase in the amount of carbonic acid exhaled in the breath.* A similar effect appears to be likewise produced in the quantity of organic matter. We were led to this conclusion from the results obtained in the case of a mechanically ventilated "half-time school," attached to one of the mills in Dundee. The children in this mill are divided into two lots, one of which works the first part of the day, and attends school the second part, the second lot alternating with the first. On two different days we made an examination of the school just before the second school began, the room having been first well ventilated for twenty minutes and the carbonic acid and organic matter determined. The children were then admitted direct from their work in the mill, and the carbonic acid and organic matter again determined at the end of thirty minutes, and again at the end of an hour. The results were as follows:—

| | First day. | | Second day.† | |
|--|----------------|-----------------|----------------|-----------------|
| | Carbonic acid. | Organic matter. | Carbonic acid. | Organic matter. |
| <i>Girls' School.</i> —Beginning of hour | 3.0 | 3.8 | 5.0 | 3.9 |
| Middle " | 9.6 | 10.7 | 12.3 | 6.2 |
| End " | 7.1 | 6.4 | 11.4 | 3.9 |
| <i>Boys' School.</i> —Beginning of hour | 4.0 | 5.1 | 3.9 | 2.9 |
| Middle " | 11.5 | 6.3 | 17.1 | 4.5 |
| End " | 11.2 | 7.8 | 15.1 | 2.9 |

6. *Cleanliness* has little or no apparent influence on the quantity of organic matter in air. (See Table, p. 96.)

* Cf. PARKES' 'Hygiene,' p. 148, where PATTENKOFER'S statement is quoted to the effect that in hard work a man evolves twice as much carbonic acid as in gentle exertion, and three times as much as during repose.

† This was the day of the great snow-storm last winter, when the quantity of organic matter in the outside air was much below the average.

SOURCES OF AND CIRCUMSTANCES AFFECTING THE NUMBER OF THE MICRO-ORGANISMS IN VITIATED AIR.

It has been shown by HESSE (*loc. cit.*) that when a room is left quiet the micro-organisms settle out in a few hours, so that the air becomes comparatively free (cf. TYNDALL'S experiments on sterilisation of air by subsidence). Hence it is clear that a certain amount of physical disturbance in a room is a condition necessary to the presence of micro-organisms in the air. It might naturally be supposed that the effects of physical disturbance would tend to obscure all other factors affecting the number of micro-organisms present in air. It is, therefore, necessary to consider first what, other things being equal, are the limits of the influence of ordinary physical disturbances on the number of micro-organisms.

The first observation bearing on this point was made at the High School. A determination was first made with the class in the room under ordinary conditions. The boys were then told to stamp with their feet on the floor for a short time. This they did with particular vigour and gusto, raising a cloud of dust which diffused itself through the room. A second determination was then made. The first determination gave 11 per litre, and the second about 150. It will be noticed that, although the increase is very great, the number found barely reaches the average in the naturally ventilated Board schools.

Such violent disturbances as that just described are, however, altogether exceptional. What is of more importance is the effect of slighter disturbances, such as occur frequently. In the same school, on another day, the boys were allowed to go out during a determination. The number found per litre was 5, as compared with an average of 1.8 in five other rooms in which the classes were sitting. The difference due to the disturbance was small as compared with the differences caused by other factors (see below). Again, in the small Chemical Lecture-room the number was actually less (1.5 per litre) at the end of a lecture than at the beginning (3 per litre). The room had only been slightly disturbed before the lecture. Again, in the Large Lecture-room the average found on three separate occasions, after an hour of a crowded popular lecture, was only 4.7 per litre. All these determinations were made before the audience left.

In the case of houses of four rooms and upwards, the rooms were classified according as the occupant rose from bed before the determination or not. The average for the former class was 12 per litre, for the latter 7 per litre. Again, in a set of observations on a block of 2-roomed houses, we started later than usual, and found that in three of the rooms visited the people were already stirring. The average in these rooms was 76 per litre, while in the other two the average was 90. The difference was due mostly to one of the former houses, which was cleaner than the rest, giving a lower number (34).

From the above results, taken in connexion with what follows, we may conclude

that the effects of minor differences as regards physical disturbance, such as cannot well be avoided in making observations in a number of rooms, are not in themselves sufficient to obscure the influence of other important factors. Nevertheless we always endeavoured to examine each class of rooms under conditions as nearly as possible the same as regards physical disturbance. In examining dwelling-rooms, it was, of course, impossible to avoid the disturbances due to our own presence in the room. But these disturbances must have been tolerably uniform, and we knew (see below) that our own persons did not act to any appreciable extent as a source of contamination.

In the cases hitherto referred to, the effects have been studied of an increase of physical disturbance apart from the simultaneous introduction of any other factor not previously in operation. A new factor, previously latent, may, however, be brought into operation by physical disturbances, as for instance when some object specially likely to give off bacteria is disturbed. For example, one of the observations at the Infirmary in the early morning was made immediately after the making of a number of beds in the ward. The number of micro-organisms found was 28 per litre, as compared with an average of 2·8 per litre in the other wards.* It was, of course, to

* We may refer here to an interesting phenomenon observed in connexion with the jelly in the tubes. It had been found that in some of the tubes crystals tended to make their appearance on the surface of the jelly after the tube had been used, and that these crystals were much more numerous and much smaller near the perforation in the cap, becoming fewer and larger towards the other end (see Plate 6. fig. 4). They thus resembled in their distribution the colonies of bacteria. We might represent diagrammatically their number and distribution as compared with the colonies of bacteria by a diagram



such as the accompanying. The inner shaded triangle represents, as regards number and distribution, the bacteria; the large triangle the crystals. These crystals only appeared in tubes made of inferior glass, apt to dissolve and crack on the surface. We have not as yet determined their composition. Probably they were composed of phosphate of lime, and were due to the lime dissolved from the glass combining with the phosphoric acid contained in the meat juice.

Of the tubes used at the Infirmary, four showed these crystals. The crystals were counted, and the corresponding numbers of crystals and colonies per litre were as follows:—

| | Crystals. | Colonies. |
|--|-----------|-----------|
| Ward 10 (5 P.M.) | 14 | 0 |
| „ 10 (5·30 A.M.) | 44 | 0 |
| „ 12 (8·30 A.M.) | 115 | 4 |
| Accident Ward (5 A.M., after some of the beds had been made) | 1600 | 28 |

In the two tubes first in the list, all the crystals were very large, like those near the cork in the last tube. Taking the numbers in the first three tubes together, the ratio of colonies to crystals is 1 : 43, while the ratio in the last tube is 1 : 57. The ratio of crystals to colonies thus appeared to correspond roughly.

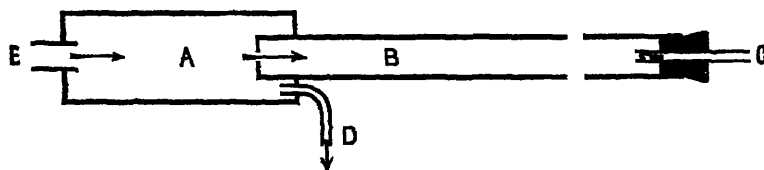
The formation of the crystals was evidently determined by solid particles falling on the jelly, just as

be expected that a large number of bacteria would be given off from bed clothes when shaken.*

In connexion with this subject some of our observations in jute mills are of interest. In each of three large rooms examined in different mills the air was loaded with jute fibres, the amount of physical disturbance being very great in each. In the air of the first, 4 bacteria per litre were found; in that of the second about 586 bacteria per litre were found, and 14 moulds; in that of the third about 12 bacteria and 248 moulds (see pp. 83-84). The latter observation was repeated with a similar result five weeks later. These observations show how small the influence of physical disturbance may be unless combined with other factors.

It is evident that the micro-organisms present in the air of an inhabited room may conceivably come (1) from the air-passages of the persons present, or (2) from their clothes and skin, or (3) may have been previously present in the room. We shall consider in succession these possible sources.

1. The air-passages might possibly give off micro-organisms in the breath. As regards this point we made the following direct experiments. A piece of very wide glass tubing, A (see diagram), of about 20 centims. length, was fitted at each end with a cap of india-rubber sheeting similar to the cap of the tube of HESSE's apparatus. Through a hole in one of the caps the end of an ordinary HESSE's tube, B, was passed, so that the part of the latter covered by its cap was completely inside the piece of wide tubing. Through another hole at the same end of the wide tube A there passed a piece of narrow glass tubing D. The cap at the other end of the wide tube was also perforated by a very short piece of glass tubing of medium width, E. The tube A having been washed thoroughly with 1 per cent. corrosive sublimate solution and allowed



to dry, the cap of the tube B was cautiously removed, and the apparatus arranged in the position described. The observer expired through the tube E, inspiring through his nose, while immediately afterwards the aspirator attached to C was set in motion. The object of D was to allow of the free escape of the breath not sucked into B. In order

crystallisation of a supersaturated solution of a salt is set up by dust particles dropping into the solution. The fact that the crystals were larger when there were few present doubtless depended on their having more jelly from which to draw during their formation. The whole phenomenon suggests a possible method for determining the number and relative rate of settling of the dust particles in given volumes of air. As the data given above tend to show, there is probably a rough correspondence, under similar conditions, between the number of solid particles suspended in air and the number of micro-organisms.

* This observation gives one a vivid idea of the manner in which an infectious disease may spread through a ward.

to prevent condensation, A was surrounded by a cloth wrung out of hot water. As the heat would have melted ordinary jelly if used for B, Agar jelly was employed instead. Another similar apparatus was prepared and placed with its tube, E, close to the face of the first observer. A second observer inspired through the tube D of the second apparatus, expiring through his nose. Unrespired air was thus drawn into the tube B under conditions as nearly as possible the same as with the expired air of the first apparatus.

Two pairs of experiments were first made when the laboratory was quiet. Each pair was carried out simultaneously, equal quantities of air being taken. The results were as follows :—

| | | |
|-------|--------------------------|----------|
| No. 1 | Respired air | 0 |
| | Unrespired air | 1 mould |
| No. 2 | Respired air | 0 |
| | Unrespired air | 1 mould. |

2 litres of air were aspirated in each case, the rate of aspiration being the same within a few seconds.

It thus appears that micro-organisms are not given off in the ordinary respiration of healthy persons—or at least not to any appreciable extent. On the contrary, those present in the air appear to stick to the mucous membrane of the nose, larynx, trachea, &c., so that the air passages practically act as filters. In order to test this more thoroughly, we made another pair of observations, the laboratory being this time full of dust stirred up by vigorous sweeping, &c. Unfortunately, however, as will be seen from the results of this experiment, the dust of this laboratory appears to contain very few micro-organisms. One litre of air was aspirated. The results were as follows :—

| | |
|--------------------------|-------------|
| Respired air | 0 |
| Unrespired air | 3 bacteria. |

These results harmonise with Professor TYNDALL'S demonstration of the optical purity of the last part of the air of each expiration. They are also in accordance with the whole of our experiments on vitiated air. Thus, by reference back to the Table (p. 86), it will be seen that, while the increase of carbonic acid runs parallel, on the whole, with the increase of organic matter, the micro-organisms first increase, and then diminish with the increase of carbonic acid. The latter diminution is also noticed if we take the rooms in the naturally ventilated Board schools, and divide them into three classes, according to the amount of carbonic acid.

| | No. of cases. | Micro-organisms. |
|--------------------------------------|---------------|------------------|
| Class 1. (Carbonic Acid 7·88–15·77). | 12 | 131 |
| „ 2. (Carbonic Acid 15·97–20·78). | 12 | 235 |
| „ 3. (Carbonic Acid 21·14–37·84). | 11 | 84 |

The lower number of micro-organisms with a higher amount of carbonic acid may probably be due to the filtration of the air through the air-passages of the scholars—not a very desirable process of purification.

A similar Table for 1- and 2-roomed houses does not show this diminution, but could not have been expected to do so, considering the small amount of carbonic acid as compared with schools. The rooms, when divided into equal classes, according as the carbonic acid was above or below a middle point, give the following results:—

| | | Micro-organisms. |
|------------|------------------------|------------------|
| One-roomed | { Carbonic acid above. | 76 |
| | { Carbonic acid below. | 57 |
| Two-roomed | { Carbonic acid above. | 38.5 |
| | { Carbonic acid below. | 38.1 |

Some experiments in the Chemical Lecture-rooms will be of interest in this connexion. At a popular lecture, during which the Large Lecture-room was crowded, and the ventilating apparatus was not in action, the carbonic acid and organic matter were, after an hour, 37.61 and 15.8 respectively (average of two determinations each). The micro-organisms, at the same time, were only 6 per litre. Again, in the Small Lecture-room (ventilation closed), just before the entry of the students (carbonic acid 5.18, organic matter 4.8), 2.5 micro-organisms per litre were found, while at the end of the lecture (carbonic acid 19.57, organic matter 11.2) 1.5 per litre were found. In another experiment in the same room (ventilation open) 3 micro organisms per litre were found at the beginning (carbonic acid 3.46, organic matter 9.2), while 1.5 were found at the end (carbonic acid 13.21, organic matter 13.1).

2. The skin and clothes of the persons present in a room at the time of an observation also occur naturally as a probable source of infection of air. That this source, however, is of much less importance than might be supposed may, we think, be shown from our observations.

In order to obtain data on this point, it is necessary to eliminate as far as possible disturbing influences arising from the condition of the room itself, particularly, as will be seen from the sequel, *habitual* bad ventilation and want of cleanliness. The Chemical Lecture-rooms are habitually ventilated mechanically, and frequently scrubbed; hence the experiments made in them are of special value in this reference. The observations in the large room were made during a course of evening popular lectures on political economy, the audience being drawn from various classes of society. There was occasional applause, which must, of course, have tended to stir up micro-organisms. The following were the results:—

| | | | Micro-organisms per litre. | |
|--------------------|----|--------------------|----------------------------|----------------|
| | | | Before lecture. | After lecture. |
| Large Lecture-room | 1. | Ventilation on . . | .. | 4 |
| | 2. | .. off* . . | .. | 6 |
| | 3. | .. on . . | .. | 3 |
| Small Lecture-room | 1. | .. off* . . | 2.5 | 1.5 |
| | 2. | .. on . . | 3 | 1.5 |

The observations at the end of the lectures were made after the audience had been in for an hour, and before they left. The fact that the numbers found were so small after the lecture shows that the influence of the bodies and clothing of persons of average cleanliness present in a room upon the number of organisms in the air is at least small as compared with the influence of other conditions.

As no micro-organisms come from the breath, those which come from persons present in a room must arise from their clothes and skin. Hence, if we take the carbonic acid as a rough measure of the total impurities arising from the persons of those present in a room, it should be a rough measure of the micro-organisms from the clothes and skin. The increase of carbonic acid does not, however, as we have seen, run parallel with increase of micro-organisms, and this supports our previous conclusion that the number of micro-organisms given off by the skin and clothes of persons actually present in a room is small as compared with those coming from other sources.

3. As the micro-organisms in the air of a room do not come to any large extent from the persons present at the time, they must come from the room itself. The circumstances in connexion with the room which are of most importance in influencing their number may be now considered.

A. *Cubic space*.—The influence of cubic space in the naturally ventilated schools which may fairly be compared with one another is traced in the Table (p. 82). It will be seen that the organisms increase with increase of cubic space up to 250 cubic feet per child, when there is no further increase. The marked diminution above 300 cubic feet, shown in the Table, depends on the observations made in a private school which was scrupulously clean. The results are therefore not comparable with those obtained in Board schools. The diminution with cubic space below 200 cubic feet will recall the similar diminution with increase of carbonic acid above a certain point. Possibly filtration through the air-passages may be again the explanation here. This is a point which we intend to investigate further.

In the case of dwelling-rooms, pp. 71-72, the micro-organisms decrease as the cubic space increases, but this must be largely due to the fact that other sanitary conditions improve as the cubic space increases. Above a thousand cubic feet there is a slight

* Only off during the lecture.

increase, not very easy to explain. In mechanically ventilated schools the micro-organisms decrease with increase of cubic space (p. 82), which is nevertheless quite in accordance with the filtration hypothesis.

B. *Cleanliness of rooms and persons habitually present in them.*—In order to show the influence of differences as regards cleanliness, we have classified the houses and schools as shown in the following Table. This Table requires no comment. It shows most conclusively the enormous influence of differences as regards cleanliness on the number of micro-organisms.

| | No of cases. | Average space per person. | Average carbonic acid. | Average organic matter | Average micro-organisms |
|---|-------------------------------|---------------------------|------------------------|------------------------|-------------------------|
| One-roomed houses | Clean | 1 | 295 | 8.0 | 18 |
| | Dirty | 7 | 200 | 9.9 | 41 |
| | Dirtier | 13 | 221 | 10.7 | 49 |
| | Very dirty | 6 | 220 | 11.0 | 93 |
| Two-roomed houses | Very clean | 2 | 273 | 12.2 | 10 |
| | Clean | 4 | 264 | 9.3 | 22 |
| | Dirty | 7 | 233 | 9.4 | 69 |
| Naturally ventilated Board schools | Cleaner | 12 | 167 | 19.7 | 91 |
| | Average cleanliness | 12 | 166 | 14.2 | 125 |
| | Dirtier | 12 | 191 | 22.5 | 198 |
| Mechanically ventilated schools and college | Cleanest | 7 | 194 | 12.5 | 3 |
| | Clean | 11 | 155 | 12.8 | 16 |
| | Less clean | 4 | 152 | 10.8 | 30 |

The houses were classified from notes made at our visits. The naturally ventilated schools were classified by the sanitary inspectors at our request.

C. *Ventilation.*—It is most important, as we shall see, to consider separately the ventilation at the time of the observation and the habitual ventilation.

a. *Ventilation at the time.*—In order to obtain data as regards its influence, we made a number of experiments on rooms provided with mechanical ventilation, examining them with it off, and again with it on, other conditions being equal as far as possible. The results are given below. The ventilation was kept off for an hour (during which the class was in the room) before the observations were made.

| | | Ventilation off | | Ventilation on. | |
|---------------------------------------|------------------|-----------------|-----------------|-----------------|-----------------|
| | | Carbonic Acid | Micro-organisms | Carbonic Acid | Micro-organisms |
| Harris Academy | Room 1 | 14.0 | 21 | 13.5 | 24 |
| | " 2 | 18.9 | 38 | 13.3 | 14 |
| | " 3 | 16.4 | 16 | 15.6 | 16 |
| | " 4* | 16.0 | 20 | | 22 |
| Small Chemical Lecture-room | | 19.6 | 15 | 13.2 | 15 |
| Large " " " " | | 37.6 | 6 | 10.8 | 3 |
| Ventilated half-time school | Boys | 15.1 | 14 | 11.6† | 47† |
| | Girls | 11.4 | 12 | 10.0† | 9† |
| Averages | | 19.6 | 16 | 12.6 | 17 |

It thus appears that ventilation by mechanical means at the time of the experiment had no appreciable effect on the number of organisms in the air of rooms, in spite of its great influence on the carbonic acid and organic matter. (See also p. 79.)

β. The influence of the habitual state as regards ventilation cannot be determined quite so satisfactorily, as it is necessary to compare results in different rooms. In order, however, to determine the effect of a few days of natural ventilation on the micro-organisms in the air of a school habitually ventilated mechanically, the artificial ventilation was stopped for a week in the half-time school previously referred to. The results were as follows:—

| | | Boys. | Girls. | | |
|-------------------------------------|---------------------|-------|--------|---------------------------------------|----|
| Previous average | | 47 | 18 | Average for the week. Boys. Girls. | |
| Ventilation off | Monday | 56 | 36 | | |
| | Tuesday | 102 | 12 | | |
| | Wednesday | 34 | 22 | | |
| | Thursday | 74 | 20 | | |
| | Friday | 52 | 6 | | |
| | Tuesday | 60 | 8 | | |
| Ventilation on since Monday morning | | | | 63 | 19 |

The observations were made at the same time each day. Unfortunately the boys were always standing and moving about at this time, hence the number of micro-organisms is made abnormally high as compared with previous results in the same room. The data obtained, however, show that the effect of having the ventilation off for a week was not appreciable in raising the number of organisms in the air. The numbers found were, in both the boys' and the girls' room, actually less in the second than in the first half of the week. These results harmonise with our observation as regards the influence of age. (See below.)

* Compared, not against the same room, but one with as nearly as possible the same space per person.

† Average of two determinations.

Although the condition as regards ventilation during short periods of time may be of slight influence on the organisms in air, yet the habitual condition seems to exercise a marked influence.

In the mechanically ventilated half-time school, just referred to, the average of all the observations made during occupation (including those with the ventilation off) was 34 per litre. The school is a very old one, and the scholars come straight from the mill to the school in their work clothes. The air in the spinning flat of the mill itself had been found to contain about 600 micro-organisms per litre. All the conditions except the habitual ventilation are thus in favour of a very high number of micro-organisms. Nevertheless, the number found is less than a fourth of the average number found in naturally ventilated Board schools (155), a sixth of that in the dirtier naturally ventilated schools, and a ninth of that in the older naturally ventilated schools.

Unfortunately, the rooms in the Harris Academy provided with mechanical ventilation are very much newer than those without it, so that the two sets of rooms are not strictly comparable. But, from the exceedingly low results in the High School (an old school), it is probable that age makes very little difference with mechanical ventilation. The average in the mechanically ventilated rooms of the Harris Academy was 16 per litre, whilst in the naturally ventilated rooms of the same school it was 117. Even if we make a large allowance for difference in age, the contrast is still very striking. The number is more than thrice as great as in the mechanically ventilated half-time school referred to above, in spite of the age of the latter, and of the scholars being much less clean.

Lastly, we may compare the naturally ventilated private school referred to previously against the High School. The cubic space and age were about the same. The private school was exceedingly clean and quiet, and there were no boys in it. Every condition was in its favour, except that the ventilation was by natural means. The average in the private school was 9·3; that in the High School 3·6. Both numbers are, of course, low.

D. In order to show the influence, if any, of age (probably as conditioned by cleanliness), we have classified the naturally ventilated Board schools as follows:—

| | No. of cases. | Micro-organisms per litre. |
|----------------------------|---------------|-------------------------------|
| Opened before 1866 | 7 | 311 |
| „ 1875-1890 | 20 | 150 |
| „ 1884-1885 | 5 | 38 |

Unfortunately, in the case of the oldest division of schools, in all but one case the children were exceptionally dirty, so that nothing can fairly be deduced from the number for this class.

We do not as yet possess sufficient data to enable us to account completely for the accumulation of micro-organisms in a room. It is possible that a room acts as a sort of trap for the particles to which bacteria or their spores are attached. This seems on the whole more likely than an actual multiplication of bacteria in the air or about the floor or furniture of a room.

RELATION OF BACTERIA TO MOULDS IN VARIOUS KINDS OF AIR.

Up to this point we have generally considered the micro-organisms as a whole, and have said but little as to the bacteria and moulds separately. It is now necessary to refer to these groups more particularly.

In this connexion the most important point is the relative proportion of bacteria to moulds in various kinds of air. This is specially interesting, because it may furnish, taken in connexion with other considerations, a valuable indication of the vitiation of air by animal and other impurities. It must be distinctly understood that the relation is that observed with jelly of the composition stated previously, and rendered faintly alkaline after heating.

In 167 out of 179 cases in which we observed the relation, the number of moulds is less than that of the bacteria. Of the exceptions, two were in the very abnormal case of the mill referred to above (p. 84). The rest were either in outside air or in very pure atmospheres (*e.g.*, four in the Infirmary). The purer the air becomes, the more nearly, as a general rule, do the bacteria and moulds become equal. Thus, in outside air in Dundee, taken in quiet open places, where there was but little traffic, there were only $2\frac{1}{2}$ bacteria on an average to each mould, whereas in the open streets in the centre of the town, during dry dusty weather, the ratio was 15 to 1. In buildings a much higher proportion usually prevails.

The following Table shows the connexion of this ratio with the general state of purity of the air in various classes of buildings :—

| | Carbonic Acid. | Organic matter. | Total micro- organisms. | Bacteria Moulds | Remarks. |
|---|-------------------|--------------------|-------------------------------|--------------------|---|
| Outside air (quiet places) | 8.9 | 8.9 | 0.8 | 2.5 | Winter. April and May. |
| " (streets) | 8.1 | 2.8 | 17.5 | 14.9 | |
| Naturally ventilated schools :— | | | | | * This number is probably too high, for many of the determinations were made during ordinary experimental lectures on chemistry, when reducing gases were possibly present. |
| Board schools | 18.6 | 16.2 | 152 | 131.8 | |
| Private school | 11.9 | 8.9 | 9 | 30.0 | |
| Mechanically ventilated schools :— | | | | | |
| General average | 12.8 | 10.1 | 16.53 | 28.5 | |
| Harris Academy (Board school) | 12.8 | 8.4 | 16.0 | 21 | |
| Half time School | 10.8 | 9.5 | 28.0 | 27 | |
| University College | 12.5 | 12.9* | 2.8 | 15.6 | |
| High School† | 15.0 | 8.9 | 3.6 | 4 | |
| Houses :— | | | | | |
| One-roomed | 11.2 | 15.7 | 60 | 49 | † The cubic space per person in this school is about three times as much as in any of the foregoing. |
| Two roomed | 9.9 | 10.1 | 45 | 20 | |
| Four and more rooms | 7.7 | 4.5 | 9 | 21 | |

The explanation of the ratio $\frac{\text{Bacteria}}{\text{Moulds}}$ increasing with the vitiation of the air is that moulds come mostly from the outside air. When the air in a room becomes vitiated the bacteria increase largely, while the number of moulds is affected to a relatively much less extent, if at all.

This is well illustrated by some observations made in the half-time school already referred to. The unoccupied room was first well ventilated by means of the fans, and the carbonic acid, micro-organisms, and organic matter determined. The children were then admitted, and the determination made at the end of half an hour, and again at the end of an hour. The results were as follows:—

| | | Carbonic Acid. | Organic matter. | Bacteria. | Moulds. |
|----------------------------|-------------------------|----------------|-----------------|----------------|---------|
| Boys' School, First day | Beginning of hour . . . | 4.0 | 5.1 | 4 | 2 |
| | Middle " . . . | 11.4 | 6.4 | Not determined | |
| | End " . . . | 11.1 | 7.3 | 56 | 2 |
| Second day . | Beginning of hour . . . | 8.9 | 2.8 | 0 | 3 |
| | Middle " . . . | 17.1 | 4.4 | Not determined | |
| | End " . . . | 15.1 | 2.8 | 14 | 0 |
| Girls' School | Beginning of hour . . . | 5.0 | 4.0 | 0 | 3 |
| | Middle " . . . | 12.3 | 6.2 | Not determined | |
| | End " . . . | 11.4 | 3.9 | 8 | 4 |

Thus in three experiments the number of bacteria was much greater at the end of the hour than at the beginning, whereas the number of moulds had remained practically constant.

The effect of stirring up dust is to increase the ratio. The bacteria are increased, while the moulds are little affected. Thus, in the High School the micro-organisms were determined just before and just after the boys had raised a cloud of dust by stamping on the floor. Before stamping, 10 bacteria and 1 mould were found; after stamping, 150 bacteria and 0 moulds. Again, in one of the wards in the Infirmary, where the beds had just been made, the ratio was $\frac{1}{4}$, while the next highest ratio observed in any of the other wards was $\frac{1}{3}$. No dust had been raised in any of the latter wards, except what was due to ordinary movements about the wards.

Relative lightness of moulds and bacteria.—The ratio of bacteria to moulds is considerably affected if the air remains quiet for any length of time, as the bacteria (or rather the particles to which they are attached), as a rule, settle out much more rapidly than moulds. In fact, the moulds settle out so slowly that we have never noticed the effects of their subsidence in the course of our observations.

The relative lightness of moulds as compared with the particles to which bacteria are attached has already been observed by HESSE ('Mitth. a. d. K. Gesundheitsamte,' vol. 2, p. 186), who found that moulds, as a rule, penetrate much further into the tubes before settling down on the jelly than bacteria do. He gives many measurements of

their actual distance. Our own observations are in accordance with his on this point. He has also made a number of very interesting observations in this connexion on the relative ease with which moulds and bacteria penetrate fine pores.

In consequence of the relative lightness of moulds, the ratio of bacteria to moulds tends to diminish when the air of a room remains at rest. We had a room kept closed for two days, after ventilation with outside air; the ratio was then found to be $\frac{.4}{.4} = \frac{1}{1}$, whereas in outside air the ratio about that time was $\frac{.25}{1}$.

The same thing is exhibited by the ratio of bacteria to moulds on still and windy days respectively, as proved by the following ratios, which are the mean results of all our available data.

| | |
|----------------------------|---|
| Still, damp days | $\frac{\text{Bacteria}}{\text{Moulds}} = \frac{.36}{.37} = 1$ |
| Windy, damp days | $\text{,,} = \frac{.63}{.5} = 1.3$ |
| Still, dry days | $\text{,,} = \frac{.7}{.27} = 2.6$ |
| Windy, dry days | $\text{,,} = \frac{10.6}{.75} = 14.1$ |

These results are all from observations in Dundee only. They show not only the effect of wind, but also that of dryness and dampness of weather. Other things being equal, there are thus fewer bacteria in the air on damp or still days than on dry or windy days. The moulds do not seem to be affected by wind or dryness to anything like the same extent.

The relative lightness of moulds and bacteria is also shown by some observations made at the top and at the foot of the Clock Tower at Westminster. The results were as follows :—

| | First observation. | Second observation. |
|---|----------------------|-----------------------|
| Top: $\frac{\text{Bacteria}}{\text{Moulds}} =$ | $\frac{2}{19} = .10$ | $\frac{2}{13} = .15$ |
| Bottom: $\frac{\text{Bacteria}}{\text{Moulds}} =$ | $\frac{7}{18} = .40$ | $\frac{18}{22} = .81$ |

At the time this experiment was made the number of moulds in the air was exceptionally large.

Standards of Purity.

It will be convenient, and, we trust, serviceable, to give at this place what we would propose as standards of purity.

The air of a dwelling-house or school must be considered bad if the following limits be exceeded :—*

| | Total. | Excess over outside air. |
|---|---------------------|-------------------------------|
| Carbonic acid { For dwelling-houses | 10 vols. per 10,000 | 6 vols. per 10,000 |
| { For schools | 13 " " | 9 " " |
| Organic matter | .. | 20 vols. oxygen per 1,000,000 |
| Total micro-organisms | .. | 20 per litre |

The ratio $\frac{\text{Bacteria}}{\text{Moulds}}$ should not exceed 30.

* The above standards are based on the following data :—

CARBONIC ACID—

- Of the 29 one-roomed houses examined, 14 contained above and 15 below 10 vols. (total),
or 9 above and 16 below 6 vols. (excess).
Of the 13 two-roomed houses examined, 5 contained above and 7 below 10 vols. (total),
or 8 above and 3 below 6 vols. (excess).
Of the 18 four (and more) -roomed houses examined, 1 contained above and 17 below 10 vols. (total),
or 1 above and 17 below 6 vols. (excess).
Of the 42 naturally ventilated school-rooms examined, 38 contained above and 4 below 10 vols. (total),
or 41 above and 1 below 6 vols. (excess).
Of the 26 mechanically ventilated school-rooms examined, 21 contained above and 5 below 10 vols.
(total), or 18 above and 8 below 6 vols. (excess).
Of the 42 naturally ventilated school-rooms examined, 31 contained above and 11 below 13 vols.
(total), or 31 above and 11 below 9 vols. (excess).
Of the 26 mechanically ventilated school-rooms examined, 9 contained above and 17 below 13 vols.
(total), or 8 above and 18 below 9 vols. (excess).
Of the 42 naturally ventilated school-rooms examined, 29 contained above and 13 below 14 vols.
(total).
Of the 26 mechanically ventilated school-rooms examined, 4 contained above and 22 below 14 vols.
(total).

ORGANIC MATTER (excess over outside air)—

- Of 29 one-roomed houses, 25 required more and 4 less than 2 vols. oxygen per million.
Of 11 two-roomed houses, 7 required more and 4 less than 2 vols. oxygen per million.
Of 13 four (and more) -roomed houses, 2 required more and 11 less than 2 vols. oxygen per million.
Of 41 naturally ventilated school-rooms, 35 required more and 6 less than 2 vols. oxygen per million.
Of 26 mechanically ventilated school-rooms, 5 required more and 21 less than 2 vols. oxygen per million.

MICRO-ORGANISMS (excess over outside air)—

- Of 28 one-roomed houses, 22 contained more and 6 less than 20 micro-organisms per litre.
Of 13 two-roomed houses, 9 contained more and 4 less than 20 micro-organisms per litre.
Of 18 four (and more) -roomed houses, 1 contained more and 17 less than 20 micro-organisms per litre.
Of 38 naturally ventilated school-rooms, 32 contained more and 6 less than 20 micro-organisms per litre.
Of 25 naturally ventilated school-rooms, 6 contained more and 19 less than 20 micro-organisms per litre.

In reference to the above standards the following remarks are necessary :—

(1.) The above limits for houses apply more particularly to sleeping-rooms.

(2.) It has been considered necessary to allow a somewhat higher limit for carbonic acid in schools than in dwelling-houses. The reasons for this are :—(a) The quantity of carbonic acid produced by respiration during waking hours is greater than when asleep, and it is therefore more difficult to maintain so low a standard in the former case.* (b) The average cubic space per person is at present considerably less in schools than in even one-roomed houses. (c) The examination of 63 different school-rooms, 30 of which were considered to be sufficiently well ventilated, shows that, even when ventilated mechanically by fans, an upper limit of 13 vols. per 10,000 (or 9 vols. in excess of outside air) is as low a one as we could reasonably expect not to be exceeded. We are fully of opinion, however, that the limit should not be fixed higher. The data on which the above opinions are based will be seen from the footnote on page 102.

(3.) The upper limit of 10 vols. per 10,000 for dwelling-houses (especially in sleeping-rooms) is the one which is usually adopted by most authorities, and this we can fully confirm. WILSON ('Handbook of Hygiene,' and quoted in PARKES, p. 115) states that in cells (in Portsmouth Convict Prison) of 614 cubic feet, always occupied, he found 7·2 vols. of carbonic acid, and that the prisoners inhabiting these cells were healthy and had a good colour. In cells of 210 cubic feet, occupied only at night by prisoners employed outside during the day, he found 10·4 vols. of carbonic acid. The occupants were all pale and anæmic.

DE CHAUMONT ('Roy. Soc. Proc.,' vol. 23, p. 187) gives 6 vols. (or 2 vols. in excess of the outside air) as the maximum amount of carbonic acid admissible in a properly ventilated space. He believes that an atmosphere ceases to be *good* when the carbonic acid reaches 8 vols. (or 4 vols. in excess); that it becomes *decidedly bad* when the carbonic acid reaches 10 vols. (or 6 vols. in excess); and that it becomes *very bad* when 12 vols. (or 8 vols. in excess) is reached. Though it would be very desirable, could this lower limit be maintained, yet from our own investigations it seems to be practically impossible, in schools at least, without involving too great a cost or using an extensive "open window" ventilation. The latter would be very objectionable, and quite inadmissible in winter. The standards proposed above are practical, and may be attained without draught, so that we may reasonably expect and demand that the air of dwellings and schools should be maintained within the limits of purity assigned above.

The lower limit for carbonic acid, proposed by DE CHAUMONT, is based as follows ('Roy. Soc. Proc.,' vol. 23, p. 187):—(1) That the air of a room should be maintained in such a state of purity that a person coming directly from the external air should

* PATTENKOFER found that in repose a man of 28 years evolved at night, when asleep, '56 cubic foot of carbonic acid, and '78 in the day-time, with very moderate exertion

perceive no trace of difference in odour between the room and outside air in point of freshness. (2) That the presence of organic matter is, on an average, perceptible to the sense of smell when the coincident carbonic acid, due to respiratory impurity, reaches 2 vols. per 10,000, or a total of about 6 vols.

In almost all the houses and schools visited we took a note of the odour perceived on entering the room; and, although as a general rule the odour was some indication of the condition of the air in the room, yet this was by no means invariably so. In some cases an extremely close and almost overpowering odour was detected when the carbonic acid amounted to only 7 or 8 volumes per 10,000, while in other cases the smell was only slight with as much as 17 volumes, and in one case as much as 20 volumes. In these latter instances the organic matter was only slightly above the limit we have allowed.

The smell is, in fact, greatly influenced by the temperature, and also by the humidity of the air, as DE CHAUMONT himself points out. The state of cleanliness of the persons in the room, and of the room itself, has a most important influence on the smell, quite independently of the amount of carbonic acid. There may also be other strongly smelling substances in a room which do not appreciably affect the chemical composition of the air. Our observations in the Infirmary wards (page 84) were very instructive in this respect. Thus, in one ward, where the excess carbonic acid was 2·9 volumes, there was a very perceptible odour. A few hours later the excess in the same ward was 3·4 volumes, but the ward felt much fresher, and the odour was barely perceptible.

For similar reasons the feeling of closeness is not a safe guide as to the amount of organic matter in a room. The combustion of gas in a room will produce a high carbonic acid and a feeling of closeness, but, as shown above, it will have little effect on the organic matter. It should be stated that DE CHAUMONT's results apply to rooms at night in which lights were not burning, whereas, in almost all the one- and two-roomed houses we visited, an oil lamp was kept burning.

The standards of purity adopted above are practical limits, which should easily be maintained by proper methods, and at not too great a cost. They are not fixed so low as might be desirable, but they are as low as practicable with the present methods of ventilation, unless, indeed, expense is no object.

As our experiments were all made between the end of November and the end of April, the standards deduced from them apply strictly only to the winter months. They might, therefore, be lowered to 8 volumes (4 volumes in excess) of carbonic acid in the case of houses, and 10 volumes in the case of schools, during warmer weather, when the injurious effects of draughts would be in great part eliminated.

(4.) The excess carbonic acid is due to respiration and combustion. The organic matter is due to respiration and the combustion of coal, oil, and possibly (to a slight extent) gas; also to dust. The carbonic acid and organic matter may, therefore, be taken as a measure of the influences contaminating the room about the time of the

observation, whereas the number of micro-organisms is largely dependent on its previous history, as shown above.

(5.) In proposing the above standards, we wish it to be distinctly understood that they should be taken in conjunction, and not singly. The carbonic acid, more especially, is not a safe guide when taken alone.

(6.) The standard for micro-organisms is for KOCH's jelly, of the composition previously stated.

So far as we are aware, a standard of purity has not previously been proposed for organic matter and micro-organisms.

THE NATURE OF THE MICRO-ORGANISMS PRESENT IN "VITIATED AIR."

On this point we purpose to say but little at present. The great majority of the colonies which appear on the jelly consist of micrococci of very various kinds and with various naked-eye appearances (see Plate 6, figs. 2 and 3). Bacilli are not nearly so common; the moulds are also of very various kinds. As was to be expected, these colonies are not always pure cultivations. In conjunction with Dr. HARE, of the Surgical Laboratory, Edinburgh University, one of us has cultivated and described some of the more characteristic and commonly occurring species. A series of inoculation and inhalation experiments with pure cultivations was also begun under Dr. HARE's direction in the same laboratory. The results of these experiments were negative in the case of the few species tried as yet.

PROBABLE INFLUENCE ON HEALTH OF THE DIFFERENT ABNORMAL CONSTITUENTS OF VITIATED AIR.

We have placed (p. 74) the results of our analyses alongside of statistics as to the death-rates in the classes of houses the air of which we examined; but it is no very easy task to determine how far differences in the death-rate are due to differences in the air breathed, and how far to other causes, such as improper or insufficient food. There is, however, abundant evidence from other sources as to the enormous influence on the death-rate of the air habitually breathed, apart from other causes (cf. PARKES' 'Hygiene,' 6th edn., p. 133). Hence we may take it as quite certain that the above differences in the death-rates in Dundee are largely due to the differences in the quality of the air habitually breathed.

As regards the influence of the separate constituents by which the air was contaminated, it is even more difficult to come to positive conclusions.* But a short discussion as to what seems probable may serve at least to give a more definite direction to one's ideas in considering the matter.

As regards carbonic acid, it seems almost certain that its presence in houses in the proportions we found could not have a sensibly deleterious effect. A slight increase

* We hope to throw further light on this point by a series of direct experiments on animals with air containing vitiating constituents separately, and not in such proportions as to cause acute poisoning.

of carbonic acid and diminution of oxygen can easily be made up for by a slight increase in the rapidity of the respirations or flow of blood through the lungs, or in the depth of the former. We know also that there are many conditions, such as adherent pleuræ or slight heart disease, which must have a very great influence on the function of respiration, and which yet do not appear seriously to affect the general health.

The case of the oxidisable organic substances in air would appear to be totally different from that of the carbonic acid. These substances, unlike carbonic acid, appear to accumulate in stagnant air until they are present in quantities as large as, or even larger than, in pure expired air. Thus the average excess of the bleaching from expired air over that of the laboratory was found to be about 7·9 in the series of observations detailed at p. 87, and the average excess in the air of naturally ventilated schools was almost exactly the same. Although, as previously remarked, there are probably other factors to be taken into account here, the figures given are sufficiently striking. They make it appear probable that increased frequency of respiration may be of no avail whatever in making up for the impurity of the air.

The facts just alluded to appear all the more striking when we consider that expired air contains about 438 volumes per 10,000 of carbonic acid, whereas the highest carbonic acid found in schools was only 37·8 volumes. It is probable that poisoning by organic substances given off by the breath and skin has a very great effect in lowering the general health and predisposing to other diseases. The deaths from "debility" and "convulsions" in infants are perhaps in considerable proportions due to sub-acute poisoning by these substances.

As regards the influence of the micro-organisms of air, it seems probable that for persons in perfect health the great majority of them are harmless. The ciliated epithelium of the respiratory passages probably sweeps them out as fast as they become entangled in the mucus with which it is bathed. Even those which have penetrated as far as the trachea and bronchial tubes are thus probably ultimately swallowed. It seems scarcely possible that any can ever reach the air-cells.

The conditions are different, however, when there is even a slight catarrh of the respiratory passages. The bacteria in air are then probably a source of considerable danger. The bacteria doubtless propagate themselves in the secretions, which are only imperfectly expelled on account of the disorganization of the epithelium, and are therefore apt to be sucked or driven into the air-cells. A condition is thus produced comparable in many respects to that in the deep part of a punctured wound. Broncho-pneumonia and further destructive changes seem a very natural consequence. It may be that few species of bacteria in addition to the bacillus of tubercle are capable of thus causing serious injury (see THAON, 'Rev. de Médecine,' December, 1885), but that bacteria in air do act in this way seems at least very probable.

This hypothesis is quite in agreement with the death-rates given above. The enormous increase in the death-rate from acute bronchitis and broncho-pneumonia is due for the most part to a simple bronchitis (caused perhaps by exposure) becoming complicated with broncho-pneumonia, which latter runs an acute and rapidly fatal course.

The very high death-rates for measles and whooping-cough in one- and two-roomed houses is due, not, to any considerable extent, to the increased frequency of these diseases, but for the greater part, especially with measles, to secondary broncho-pneumonia. They are diseases which the majority of children of all classes have some time or other. During even a mild attack the respiratory passages are in a condition which, as explained above, makes them specially liable to be attacked by micro-organisms. Hence it is natural that broncho-pneumonia should appear in proportion to the contamination of the air breathed by the patient. In a ward in a children's hospital, where the ventilation was bad, THAON (*loc. cit.*) has observed that the mortality is three times as great as in another with good ventilation.

In scarlet fever there is no bronchial catarrh, hence the micro-organisms of air are probably not a special source of danger. We have inserted the death-rates from scarlet fever in the Table, although the number of deaths is too small for any definite conclusions to be drawn from them. The death-rate for the year was twice as great in the houses of three rooms and upwards as in the one- and two-roomed houses. If, however, we take the mean of this and the previous year, for which we also possess data as regards scarlet fever, the death-rate (31 deaths in all) is about a third greater in one- and two-roomed houses. This increase is not in proportion to the increase (two-thirds) in the general death-rate in the lower class of houses, and does not compare with the increase from measles, and, to a less marked extent, whooping-cough.

It will be of interest to reproduce here a valuable Table prepared by Professor MAX C-RUBER ('Wiener Med. Wochenschrift,' December 26th, 1885) from KÖRÖSI's statistics of the town of Buda-Pesth for the years 1879-82. KÖRÖSI had compared the death-rates in the lowest class of rooms ("cellars") with the death-rates in the rest of the town for various diseases, and the Table shows the percentage increase or decrease in the death-rates.

| | Percentage increase or decrease in death-rate |
|--------------------------------|--|
| Measles | +159 |
| Whooping-cough | +100 |
| Scarlet fever | - 8 |
| Croup and diphtheria | + 11 |
| General death-rate | + 35 |

The death-rate from scarlet fever here shows an actual decrease in the lowest class of dwelling. It seems just possible that this low death-rate may to some extent be accounted for by the same reasons as those for which artificially inoculated small-pox is less fatal. We cannot doubt that scarlatina is more common in the lower class of houses, and that, *ceteris paribus*, it is more fatal.

With diphtheria and croup, broncho-pneumonia is of exceedingly frequent occur-

rence, it is true (found in 90 per cent. of the fatal cases of diphtheria, according to THAON, *loc. cit.*) But here the chances of broncho-pneumonia are about equal, whatever the number of micro-organisms in the air, because in these diseases the broncho-pneumonia is due to the specific poison of the disease, and caused by the spread downwards of infection from the throat. In diphtheria the patches of broncho-pneumonia are, according to THAON, full of the same organisms as are present in the false membrane, and which produce artificial diphtheria in animals (LOEFFLER, 'Mittheilungen a. d. K. Gesundheitsamte,' vol. 2, p. 421). The death-rates both in Dundee and Buda-Pesth are in consistence with this hypothesis.

The curious relation in the death-rate from phthisis appears to depend on the mutual interaction of constitutional predisposition and bad hygienic conditions. The mere fact that, the worse the hygienic conditions, the more do children who are weakly die off in childhood, does not altogether account for the differences in the death-rate from phthisis. For, in spite of this survival of the fittest, the general death-rate above 20 increases steadily from the better to the worse houses (including Infirmary and Poor-houses), although the increase is nothing like so great as it is for children. Were phthisis due merely to the influence of bad hygienic conditions, we should, therefore, still expect the death-rate to increase up to the two- and one-roomed houses. As a matter of fact, although those under good hygienic conditions suffer by far the least from phthisis, yet it tells most heavily on those under only moderately bad hygienic conditions, and this is the case even when all the deaths in the Infirmary and Poor-houses are set down against those under the worst hygienic conditions, *i.e.*, those in the one- and two-roomed houses. This would seem to indicate a predisposition to tubercular disease in a certain proportion of the individuals, quite apart from general constitutional weakness. Under good hygienic conditions the great majority of these will escape altogether. Under medium conditions a large proportion will survive the tubercular diseases of childhood, but only to fall victims to phthisis later on in life. Under the worst conditions most will die of tubercular disease during childhood. So few will survive that, although a larger proportion of them will die than of the survivors under medium conditions, yet the death-rate from phthisis on the whole population in the worst houses will be less.

The fact that the mortality from croupous pneumonia increases so markedly from four- to two-roomed houses, and out of all proportion to the death-rate above childhood, is an important confirmation of the theory of its being caused by micro-organisms. The fact that it is rather less fatal in one- than in two-roomed houses is due probably to the fact that it is to a very large extent treated in the Infirmary, where very many recover who would certainly die at home.

REMEDIES.

It is not within the scope of this paper to discuss at length the methods which should be employed for maintaining a reasonably pure atmosphere in schools and in dwellings; but the following suggestions, as the result of experience, may not be without some value.

(1.) As our results show, the state of the air in the Board schools in Dundee is extremely bad, and urgently needs improvement. Doubtless the schools in other towns are in a similar condition. The symptoms ascribed to overpressure, which has been complained of so extensively of late, are probably largely due to the defective ventilation of the schools. Defective ventilation weakens and depresses the energies so that a child certainly cannot gain the full advantage of its education under a bad system of ventilation. It therefore behoves School Boards to pay much more attention than hitherto to the ventilation of their schools.

A sufficiently pure air in schools appears to be attainable only by mechanical ventilation. It is true, the necessary conditions of purity may also be got by the use of open-window ventilation; but then, in winter at least, the ill-effects of draughts are probably greater than those due to insufficient ventilation.

Of the systems of mechanical ventilation, it is better to blow air into the room, and allow it to find its own way out (preferably up special shafts), than to ventilate by extraction. Draughts are more easily avoided by the former method. But the great objection to the suction method is that a partial vacuum tends to be produced, which would greatly accelerate the entry of sewer-gas into the room from any defective drains, whereas the "blow-in" method has the positive advantage of producing the opposite effect. By blowing warm air into a room a much more uniform and higher temperature may be attained during winter; and this method is also independent of the state of the weather. All parts of the room are more thoroughly heated and ventilated than under the natural system. The air should be blown in under a low velocity through sufficiently large upright shafts, in order to avoid draughts.

(2.) In regard to houses, mechanical ventilation is, of course, out of the question, but very satisfactory results may be obtained in the case of a large block of buildings let out on flats in single- or double-roomed houses, as is largely the case in Scotch towns, by having a large open-air space or landing on each flat, and provided with open wire-grated windows without glass, so that a good current of fresh air may be maintained along the passages and staircases, whereby a pure, instead of an already vitiated, air enters and supplies the various rooms.

This was shown in a very marked manner in a large block of such one-roomed houses in Dundee. This block was eight stories high, covered an area of 555 square yards, and contained 136 separate one-roomed and four two-roomed dwelling-houses. Owing to bad trade, only the four lower flats are now occupied, but a few years ago all the houses were tenanted, and then had a population of about 700, or about three

quarters of a square yard of ground to each person. It is, in fact, the largest and closest-packed block in Dundee. Formerly the ventilation was very bad, but by order of the sanitary authorities one room on each flat was thrown open as an air space, and the glass removed from the windows and replaced by open wire grating. The average temperature in this block was 54° , or only 1° below the average of all the one-roomed houses we examined, although the outside air at the time was 5° lower on the average than on the nights when the other one-roomed houses were examined. The analytical results obtained were as follows :—

| | General average of 1-roomed houses | Block of buildings referred to | | | |
|-------------------------------|--|--------------------------------|-----------------------|----------------------|-----------------------|
| | | House on 4th flat | House on 3rd flat. | House on 3rd flat | House on 2nd flat. |
| Space per person | 212 cubic feet | 193 | 169 | 193 | 225 |
| Temperature | 55° Fahr. | .. | 52 | 53 | 56 |
| Carbonic acid | 11.2 | 8.6 | 7.9 | 8.8 | 10.0 |
| Organic matter (excess) . . . | 6.7 | 4.0 | 3.8 | 3.0 | 3.3 |
| Total micro-organisms . . . | 60 | 21 | 95 | 45 | 53 |

From this it will be seen that the open-air spaces have a marked effect in improving the condition of the air in such houses as those referred to. Since this alteration has been made a very marked improvement has taken place in the health of the inhabitants of this block of buildings.

(3.) The practice adopted in almost all small houses of keeping a lamp burning during the night is one to be deprecated, as it must add very considerably to the contamination of the air, especially in one-roomed houses, in which the cubic space per person is so small.

(4.) Cleanliness, both of the person, and more particularly of the dwelling or school, is of the very utmost importance in maintaining the purity of the air as regards micro-organisms, and one which from this point of view has not been previously advocated.

(5.) Though far from depreciating the beneficial effect of abundant air space, yet we think that the frequency with which the air in a room is changed is a far more important point to be attended to in providing a pure atmosphere.

(6.) Ventilation by mere diffusion should never be depended on alone, for, though it may remove a considerable portion of the carbonic acid, it has, probably, but little effect in reducing the organic matter and micro-organisms.

(7.) It is most important that the windows in houses and schools should be made to open widely, so that at intervals a good current of air may be sent through the room. This would be very effective in removing the organic matter and micro-organisms.

In conclusion, we have to express our best thanks to Miss ETTA JOHNSTONE, of

University College, for her paintings; to Mr. KINNEAR, Chief of the Sanitary Department, for his kindness in constantly furthering our work; to the Head Masters of the various schools we examined, and particularly to the Rectors of the High School and Harris Academy. Many other gentlemen have also given us valuable assistance, for which we are indebted. The photograph we owe to the kindness of Professor STEGGALL.

SUPPLEMENTARY NOTE.

(Added December, 1886.)

On dividing the naturally ventilated schools we examined into two classes, according as they were heated and ventilated by fires or by hot pipes respectively, we obtained the following results. The data for mechanically ventilated schools are added for comparison.

| Description of School. | No of rooms examined. | Carbonic acid. | Organic matter. | Total micro-organisms. |
|--|-----------------------|----------------|-----------------|------------------------|
| Ventilated mechanically, and heated by hot air blown into the rooms | 20 | 12·3 | 10·1 | 16·5 |
| Heated by fires, and ventilated in the ordinary way | 18 | 16·9 | 15·7 | 169· |
| Heated by hot pipes in the room itself, and ventilated by windows, ventilators in the room, and in some cases by a few TOBIN'S tubes | 21 | 20·0 | 16·5 | 92· |

The above Table shows that with fires the carbonic acid was considerably and the organic matter slightly less than with hot pipes, while the number of micro-organisms was very much larger.

The above average for micro-organisms in schools with fires does not include the four denominational school-rooms. If we include them, the average is 241. The number 169 for micro-organisms includes all the Board schools; but those with fires have an average age of about fourteen years, whereas those with hot pipes average only about seven years. If, however, we take only the schools built between 1875 and 1880, the averages are as follows, and give practically the same results as regards the large excess of micro-organisms in schools with fires.

| | No. of rooms examined. | Average age. | Total micro-organisms. |
|-------------------------------------|------------------------|--------------|------------------------|
| Schools with fires, built 1875-80 . | 7 | years 2·9 | 171· |
| „ hot pipes, „ . | 13 | 3·5 | 108· |

V. *A New Method for the Quantitative Estimation of the Micro-organisms present in the Atmosphere.*

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Communicated by Professor FRANKLAND, D.C.L., LL.D., M.D., F.R.S.

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THE quantitative estimation of the micro-organisms present in air is a problem which has attracted the attention of many experimenters since it received its first impetus at the hands of PASTEUR * twenty-six years ago.

The method originally employed by PASTEUR was of a very simple character, and consisted in preparing vacuous flasks of definite ($\frac{1}{2}$ litre) capacity, each containing a small quantity of nutritive liquid; the air in the flask was removed by boiling the liquid, and the open extremity was then sealed with the blowpipe. Such sealed flasks could then be preserved for an indefinite period of time without undergoing change. By breaking off its sealed extremity, however, a certain volume of air rushes into the flask, carrying with it any microbes that it holds in suspension; the flask is again immediately sealed, and if any microbes have gained access the liquid will, after suitable incubation, suffer visible alteration. On opening a number of such flasks in various places, PASTEUR found that the proportion of them which suffered alteration was dependent upon the locality in which the experiment was performed. Thus, out of twenty such flasks exposed in the country at a distance from all habitations, eight became contaminated; of twenty others exposed on the first heights of the Jura Mountains, five became infected; whilst of twenty others exposed on the Montanvert (2000 metres high) one alone broke down.

TYNDALL,† in order to obtain an idea of the distribution of micro-organisms in the air, constructed a square table provided with 100 apertures, in which 100 test-tubes, each containing sterile culture-fluid, could be placed. This battery of tubes was then exposed to the air under examination, and the fate of the tubes watched from day to day. It was found that the tubes did not all suffer change simultaneously, but that the date of their alteration was dependent upon their position; moreover, the changes taking place in the different tubes varied according to the particular kind of organism

* 'Compt. Rend.' vol. 51, 1860, p. 348.

† 'Phil. Trans.,' 1876, Part I.

with which the individual tube had become infected, and thus, in a measure, this apparatus foreshadows some of the great advantages to be obtained from the use of a solid culture-medium in which each individual organism gives rise to a separate colony.

FREUDENREICH and MIQUEL* have further elaborated processes for the quantitative distribution of micro-organisms in air, their researches having led them to adopt the following method, which has been largely used in the well-known experiments at the observatory of Montsouris.

This method consists essentially in aspirating a definite volume of air through a plug of sterilised glass-wool, which is then agitated with a definite volume of sterilised water, so as to distribute the collected organisms in the latter. This water is then divided into a number of equal parts, each of which is added to a tube, flask, or bulb containing sterilised broth. The volume of water with which the plug is mixed must be so selected that, when divided into the given number of equal parts, the micro-organisms are so attenuated that *only a portion* of the total number of inoculated tubes become infected. If only a portion of the total number of tubes become thus infected, MIQUEL argues that the organisms were distributed at such wide intervals in the water that not every one of the parts into which the latter was divided contained even a single organism, and that, therefore, those broth-tubes which suffered alteration through the inoculation of such a part must have received only a single organism, and, thus, that the number of culture-tubes which break down is identical with the number of organisms communicated to the water by the plug.

KOCH† first applied a solid nutritive medium to the examination of air for micro-organisms by exposing glass dishes containing a nutritive solid surface, such as peptone-gelatine, potatoes, &c. Upon this surface the aerial microbes become deposited, and, after suitable incubation, give rise to colonies which can be counted and further examined.

This method, which forms an entirely new departure in the examination of air, was further developed by HESSE,‡ who, by aspirating air through wide glass tubes of about 2 feet 6 inches in length and 1.5 inch diameter, coated internally with peptone-gelatine, found that, provided the current of air was not too rapid, practically the whole of the suspended organisms were deposited on the bottom of the tube in the first half or two-thirds of its length. The remarkable phenomenon of the rapid subsidence of organisms in comparatively still air, upon which this method depends, is similar to that previously observed by TYNDALL§ in his well-known sterile chambers coated with glycerine.

* 'Annuaire de l'Observ. de Montsouris,' 1884, p. 533.

† 'Mittheil. Kaiserl. Gesundheitsamte,' vol. 1, 1881, p. 32.

‡ Ditto, vol. 2.

§ TYNDALL, *loc. cit.*

Advantages and Disadvantages of Methods in Vogue.

Of the methods mentioned above, the two most generally in use at the present time are those of MIQUEL and of HESSE. The former finds most favour with those experimenters who still work principally with liquid media, whilst HESSE's method is preferred by those employing a solid material for the cultivation of micro-organisms.

There are many objections to MIQUEL's process, sufficiently obvious from the above description. Thus, in the first place, each individual experiment is attended with great expenditure of time, trouble, and material, for the water with which the glass-wool plug is mixed has to be divided amongst a large number (ten, thirty, forty, or more) of culture-tubes in one single experiment, so that the preparation of these alone for an extensive series of investigations must become an intolerable burden. A still weaker point in the method lies in the necessity of so selecting the quantity of water with which the glass-wool plug is mixed that, when divided into the given number of equal parts for inoculation into the culture-tubes, *each part shall not contain more than one organism*, for, as pointed out above, it is assumed in the subsequent estimation of the number of organisms present that each culture-tube which suffers alteration has succumbed through the introduction of a single organism. Should, therefore, the quantity of water taken for mixing be wrongly proportioned and the whole of the inoculated culture-tubes break down, the experiment is rendered worthless, as no calculation with regard to the number of micro-organisms can then be made. Again, the assumption that each tube suffering alteration does so in consequence of the introduction of a single organism must be accepted with much reserve, more especially as the difficulty of equally distributing the organisms in the water (and upon this equal distribution the process is absolutely dependent) is rendered particularly difficult through the presence of the suspended particles of glass-wool, and of this difficulty I shall furnish experimental proof later on.

Owing to the above objections to MIQUEL's process, and in consequence of the obvious advantages to be secured by working with a solid nutritive medium, I have myself adopted HESSE's method for carrying out a number of experiments on the distribution of micro-organisms in air, the results of which were communicated to the Society in June last.* This method possesses the great advantage that the tubes, after preparation in the laboratory, can be transported to the place where the experiment is to be performed, and that there are no further operations requiring special appliances. In hot weather, however, there is considerable difficulty in carrying the tubes about, and I had to devise special precautions, which I have described in another communication, for preventing the melting of the gelatine film in experiments performed in direct sunshine. After a very short acquaintance with HESSE's apparatus, however, I became convinced that it was a matter of great consequence in which direction the tube was placed with regard to the wind, and that, if the open extremity

* 'Roy. Soc. Proc,' vol. 40, p. 509

of the tube pointed *towards* the wind, the number of organisms deposited in the tube was greater than when pointing *away* from the wind. It is a matter of some surprise to me that no reference to this point is made in HESSE's original paper, and that he makes no special recommendation as to the relationship which should exist between the direction of the wind and that of the tube. In my experiments with his apparatus I have made a practice of directing the open end of the tube at an angle of 135° away from the wind, so that the latter does not blow into the tube, whilst, at the same time, the experimenter does not intervene between the wind and the open end of the tube. The direction of the wind is, however, very rarely so constant that this angle can be preserved throughout an entire experiment, which usually extends over 1 hour or even more; commonly the direction of the wind is subject to considerable and rapid local variations, whilst, sometimes, a complete change of direction takes place, in consequence of which I have, in some cases, had to reject the results. In my later experiments I have generally exposed side by side with the tube through which the air was aspirated a second similar tube by way of control, and I have ascertained that the number of colonies making their appearance in the control-tube frequently amounts to a large fraction of the number found in the tube through which the air has been aspirated. In illustration of this, I may quote the following results obtained in this manner:—

Experiments in Open Air.

HESSE's Method with Control-tube.

I. *Gardens of Natural History Museum.* June 9th, 1886.

| | |
|------------------------------------|---------------|
| 12 litres of air yielded | 158 colonies. |
| Control-tube | 54 „ |
| Wind moderate. | |

II. *Ditto.* June 10th, 1886.

| | |
|------------------------------------|--------------|
| 11 litres of air yielded | 20 colonies. |
| Control-tube | 6 „ |

In this experiment, which was after heavy rain, the wind was very slight.

III. *Roof of Science Schools, South Kensington Museum.* June 18th, 1886.

| | |
|------------------------------------|--------------|
| 12 litres of air yielded | 12 colonies. |
| Control-tube | 8 „ |
| Wind slight. | |

IV. *Ditto.* June 22nd, 1886.

| | |
|------------------------------------|--------------|
| 12 litres of air yielded | 53 colonies. |
| Control-tube | 11 „ |

Wind moderately strong.

V. *Roof of Science Schools, South Kensington Museum.* June 25th, 1886.

12 litres of air yielded 114 colonies.

Control-tube 34 ..

Wind moderately strong.

VI. *Ditto.* June 28th, 1886.

12 litres of air yielded 49 colonies.

Control-tube 29 ..

Wind moderate, but variable.

VII. *Ditto.* Same day.

11 litres of air yielded 53 colonies.

Control-tube 15 ..

Wind moderate, but not quite so variable as in previous experiment ;
increased very much in strength at close of experiment.VIII. *Ditto.* June 29th, 1886.

12 litres of air yielded 45 colonies.

Control-tube 11 ..

Wind very gentle and fairly constant.

IX. *Ditto.* June 30th, 1886.

10 litres of air yielded 75 colonies.

Control-tube 15 ..

(approximately)

(Owing to strength of wind, the open extremities of the tubes were
directed quite away from the wind.)

Wind very considerable in strength.

X. *Ditto.* July 2nd, 1886.

12 litres of air yielded 51 colonies.

Control-tube 10 ..

Wind gentle throughout the greater part of experiment.

XI. *Ditto.* July 6th, 1886.

12 litres of air yielded 78 colonies.

Control-tube 48 ..

Wind very considerable.

XII. *Ditto.* July 9th, 1886.

12 litres of air yielded 59 colonies.

Control-tube 22 ..

Wind strong.

XIII. *Roof of Science Schools, South Kensington Museum.* July 12th, 1886.

12 litres of air yielded 72 colonies.

Control-tube 27 „

Wind slight.

XIV. *Ditto.* July 21st, 1886.

12 litres of air yielded 129 colonies.

Control-tube 32 „

Wind very gentle during the first half, but increasing considerably at end of experiment.

XV. *Ditto.* August 3rd, 1886.

10 litres of air yielded 105 colonies.

Control-tube 50 „

Wind gentle, but variable in direction.

XVI. *Ditto.* September 24th, 1886.

12 litres of air yielded 71 colonies.

Control-tube 36 „

Wind very gentle, but variable both in strength and direction.

XVII. *Ditto.* September 25th, 1886.

12 litres of air yielded 32 colonies.

Control-tube 16 „

Wind very gentle.

XVIII. *Ditto.* October 19th, 1886.

9 litres of air yielded 31 colonies.

Control-tube 2* „

Wind slight, constant in direction.

XIX. *Ditto.* October 22nd, 1886.

11 litres of air yielded 18 colonies.

Control-tube 10 „

Wind gentle, but variable both in strength and direction.

XX. *Ditto.* October 25th, 1886.

10 litres of air yielded 68 colonies.

Control-tube 17† „

Wind very strong, but fairly constant in direction.

XXI. *Ditto.* October 29th, 1886.

12 litres of air yielded 35 colonies.

Control-tube 6 „

Wind gentle, but somewhat variable in direction, and more so in strength.

* A liquefying colony had probably obliterated some of the other colonies.

† This number is doubtless too small, owing to tube being considerably obliterated by a liquefying colony.

Experiments in Buildings.

- I. *Natural History Museum.* June 14th, 1886 (Whit-Monday)
- | | |
|------------------------------------|---------------|
| 10 litres of air yielded | 280 colonies. |
| Control-tube | 49 „ |
- II. *Ditto.* Same day.
- | | |
|------------------------------------|---------------|
| 10 litres of air yielded | 267 colonies. |
| Control-tube | 39 „ |
- III. *Chemical Laboratory, Science Schools, South Kensington.* October 15th, 1886.
- | | |
|------------------------------------|--------------|
| 10 litres of air yielded | 30 colonies. |
| Control-tube | 5 „ |
- IV. *Ditto.* October 16th, 1886.
- | | |
|-----------------------------------|--------------|
| 9 litres of air yielded | 13 colonies. |
| Control-tube | 2 „ |
- V. *Ditto.* October 27th, 1886.
- | | |
|------------------------------------|--------------|
| 10 litres of air yielded | 32 colonies. |
| Control-tube | 2 „ |
- N.B.—The room must have been very free from aerial currents, as windows and door were closed and nobody was moving about.

The two series of experiments recorded above show that the number of organisms gaining access to HESSE's tubes, irrespectively of aspiration, is greater in out-door than in experiments within doors, this difference being obviously due to the more disturbed state of the external air. Thus, in the 21 experiments made in the open air, the control-tube contained, on an average, 0·36 of the number of colonies found in the tube through which air was aspirated, whilst in the experiments made in-doors the proportion so found did not amount to more than 0·16. This introduces a point of great difficulty in the interpretation of the results obtained with HESSE's apparatus, for it is obvious that the number of colonies obtained in the tube through which air is drawn is in excess of the number of organisms suspended in the air actually aspirated; whilst, on the other hand, it is very probable that the number of colonies obtained in the control-tube is greater than the number of excess-organisms in the tube through which air is drawn. Thus, in the first open-air experiment recorded above, the number 158 is obviously too high, whilst 158—54 is probably too low, the truth lying somewhere between the two; but to this point I shall return later on.

New Process.

Already in the winter of 1885 I made some preliminary experiments on the bacterioscopic examination of air by aspirating a known volume through sterile glass

or cotton wool, and then transferring the latter to nutrient gelatine. In consequence of numerous difficulties with which I was then met, I abandoned this method for Hesse's apparatus, and it was not until I became convinced of the imperfections in the latter, to which I have referred above, that I again endeavoured to elaborate a new process.

I. Gelatine Plate Method.—In the first instance, a known volume of air was drawn by means of a hand-pump through a small sterilised glass tube, about 4 inches long and $\frac{1}{8}$ inch in diameter; this glass tube was fitted with a plug constructed of either a stratum of fine glass-powder between two layers of glass-wool or a stratum of fine sugar-powder between two layers of glass wool, which had been soaked in a strong solution of cane-sugar and subsequently dried. The tube was slightly constricted behind the plug, so as to prevent the latter being drawn through by the suction of the pump. This plug, after use, was pushed by means of a sterilised wire into a small sterilised stoppered bottle, and a definite volume (5, 10, or 20 c.c.) of sterilised water or broth added, and the whole violently shaken for some 5 or 10 minutes until the plug had become thoroughly disintegrated. In the case of the sugar plugs, this was rapidly accomplished, owing to the greater part passing into solution and leaving only the glass-wool skeleton behind in a fine state of division. The glass plugs are somewhat more difficult to break up.

Definite quantities of the liquid were now taken out by means of a sterilised pipette, and added to peptone-gelatine, with which plates were poured in the ordinary way. The colonies subsequently making their appearance on these plates were counted as in the examination of water, and thus the number of organisms present on the whole plug was calculated. The following details of some experiments will serve to illustrate the method of procedure:—

I. Science Schools, South Kensington Museum. Laboratory window (west front, 3rd floor). July 13, 1886. Wind, S.W.; rain on previous day.

Volume of air aspirated = 132 litres.

Construction of plug (single).—Glass-powder between two layers of glass-wool.

Plug mixed with 20 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation:—

Colonies, 61, 60, 47, 41 . Average = 52.25 colonies.

132 litres of air yielded $\therefore 52.25 \times 20 = 1045$ „

10 „ „ 79 „

II. Laboratory window (same). July 14, 1886. Wind, W. by N.W.; strong, much dust blowing in street below.

Volume of air aspirated = 106 litres.

Construction of plug (single).—Glass-powder between two layers of glass-wool.

Plug mixed with 20 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation:—

Colonies, 121, 62, 64, 58. Average = 76 colonies.
 Control-plate gave 5 „
 106 litres of air yielded $\therefore 71 \times 20 = 1420$ „
 10 „ „ 134 „

III. *Laboratory window* (same). July 15, 1886. Wind, W. by S.W., varying to N.W., fairly strong.

Volume of air aspirated = 92 litres.

Construction of plug (single).—Glass-powder between two layers of glass-wool.

Plug mixed with 20 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation:—

Colonies, 37, 25, 53, 36. Average = 38 colonies.
 1 c.c. of sterilised water gave . . . 6 „
 92 litres of air yielded $\therefore 32 \times 20 = 640$ „
 10 „ „ 70 „

IV. *Laboratory window* (same). July 16, 1886. Wind, W. by N.W., strong; dust blowing about in street below.

No. 1. Morning. *Volume of air aspirated* = 106 litres.

Construction of plug.—Loose. Powdered glass ($\frac{1}{16}$ " thick) between two layers of glass-wool.

Rate.—About 5 strokes* per minute.

Plug mixed with 20 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation:—

Colonies, 28, 21, 26 . . . Average = 25 colonies.
 Sterilised water only gave on control-plate 3 „
 106 litres of air yielded $\therefore 22 \times 20 = 440$ „
 10 „ „ 42 „

Laboratory window (same). July 16, 1886.

No. 2. Afternoon. *Volume of air aspirated* = 87 litres.

Construction of plug.—Tight. Powdered glass ($\frac{3}{8}$ " thick) between two layers of glass-wool.

Plug mixed with 20 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation:—

Colonies, 18, 25 . . . Average = 21.5 colonies.
 The above control-plate applies equally
 to these plates.
 87 litres of air yielded $\therefore 18.5 \times 20 = 370$ „
 10 „ „ 43 „

N.B.—The plugs in these two experiments were not further treated until the

* Nine strokes of air-pump employed are equivalent to $\frac{1}{16}$ cubic foot = 2.86 litres.

following day. The results are, therefore, doubtless too low, and only comparable *inter se*.

V. *Laboratory window* (same). July 19, 1886. Wind gentle, S. by S.E. Roads and pavements quite wet. Raining at time; tube, however, protected by angle of building

No. 1. Morning. *Volume of air aspirated* = 119 litres.

Construction of plug.—Glass-wool and glass-powder ($\frac{1}{8}$ " thick).

Rate.—About 3·6 strokes per minute.

Plug mixed with 20 c.c. of sterilised water, of which 1 c.c. gave on plate-cultivation :—

Colonies, 36, 48, 45 . . . Average = 43 colonies.

Two control-plates with same sterilised

water gave 2, 4 colonies . Average = 3 „

119 litres of air yielded $\therefore 40 \times 20 = 800$ „

10 „ „ 67 „

No. 2. Afternoon. *Volume of air aspirated* = 119 litres.

Construction of plug.—Sugared glass-wool and sugar-powder ($\frac{1}{8}$ " thick), but plug not otherwise so tight as in No. 1.

Rate.—About 5·6 strokes per minute.

Plug mixed with 20 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation :—

Colonies, 31, 48, 31 . . . Average = 36·7 colonies.

The above control-plates apply equally to this experiment.

119 litres of air yielded $\therefore 33·7 \times 20 = 674$ „

10 „ „ 57 „

N.B.—The plugs in these experiments were not treated until the following day, and the results are, therefore, doubtless too low, and only comparable *inter se*.

VI. *Laboratory window* (same). July 20, 1886. Wind, due S., moderately strong. Very heavy rain on previous night.

Volume of air aspirated = 119 litres.

Construction of plug.—Sugared glass-wool and sugar-powder.

Rate.—About 4·9 strokes per minute.

Plug mixed with 20 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation :—

Colonies, 26, 25, 35 . . . Average = 28·7 colonies.

Two control-plates with sterilised water

gave colonies 4, 2 . . . Average = 3 „

119 litres of air yielded $\therefore 25·7 \times 20 = 514$ „

10 „ „ 43 „

VII. *Laboratory window* (same). July 21, 1886. Wind, S. by S.E., fairly strong.

Dust was being blown about in street below. $23^{\circ}9$ C.

Volume of air aspirated = 119 litres.

Construction of plug.—Sugared glass-wool and sugar-powder.

Rate.—About 3.5 strokes per minute.

Plug mixed with 20 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation :—

Colonies, 77, 63, 65. . . . Average = 68.3 colonies.

Control-plates with sterilised water gave

colonies 12, 4 Average = 8 „

119 litres of air yielded $\therefore 60.3 \times 20 = 1206$ „

10 „ „ 101 „

VIII. *Laboratory window* (same). July 22, 1886. Wind, S.W., strong, variable in strength and direction. $23^{\circ}9$ C.

No. 1. *Volume of air aspirated* = 119 litres.

Construction of plug.—Sugared glass-wool and sugar-powder.

Rate.—About 5.1 strokes per minute.

Plug mixed with 20 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation :—

Colonies, 97, 55, 61 Average = 71 colonies.

Control-plate with sterilised water gave 3 „

119 litres of air yielded $\therefore 68 \times 20 = 1360$ „

10 „ „ 114 „

No. 2. Wind stronger than in No. 1, and much dust blowing about.

Volume of air aspirated = 119 litres.

Construction of plug.—Glass-wool and glass-powder.

Rate.—4.7 strokes per minute.

Plug mixed with 20 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation :—

Colonies, 107, 103, 110 Average = 106.7 colonies.

Control-plate with sterilised water gave 6 „

119 litres of air yielded $\therefore 100.7 \times 20 = 2014$ „

10 „ „ 169 „

IX. *Laboratory window* (same). July 26, 1886. Wind, W. by S.W. Copious rain during previous day and night, but roads and pavement dry at time of experiment. 20° C.

No. 1. *Volume of air aspirated* = 119 litres.

Construction of plug.—Sugared glass-wool and sugar-powder.

Rate.—3.8 strokes per minute.

Plug mixed with 20 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation :—

Colonies, 26, 21, 21 . . . Average = 22·7 colonies.

Plate similarly prepared from control-

plug gave colonies 6, 3 . Average = 5 „

119 litres of air yielded $\therefore 17\cdot7 \times 20 = 354$ * „

10 „ „ 30 „

No. 2. Afternoon. Heavy shower since morning.

Volume of air aspirated = 119 litres.

Construction of plug.—Similar to above.

Rate.—8 strokes per minute.

Plug mixed with 20 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation :—

Colonies, 4, 12, 9 . . . Average = 8·3 colonies.

Plates similarly prepared from control-

plug gave colonies 1, 0 . Average = 1 „

119 litres of air yielded $\therefore 7\cdot3 \times 20 = 146$ „

10 „ „ 12 „

X. *Roof of Science Schools*. July 29, 1886. Wind, S. by S.W., gentle. No previous rain. Roads watered. 21°·3 C.

No. 1. Morning. *Volume of air aspirated* = 119 litres.

Construction of plug.—Glass-wool and glass-powder.

Rate.—8 strokes per minute.

Plug mixed with 20 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation :—

Colonies, 28, 19, 16 . . . Average = 21 colonies.

Plates similarly prepared from control-

plug gave colonies 5, 4 . Average = 4·5 „

119 litres of air yielded $\therefore 16\cdot5 \times 20 = 330$ „

10 „ „ 28 „

No. 2. Afternoon. *Volume of air aspirated* = 60 litres.

Construction of plug.—Same as No. 1, more closely packed.

Rate.—4 strokes per minute.

Plug mixed with 10 c.c. of sterilised distilled water, of which 1 c.c. gave on plate-cultivation :—

Colonies, 19, 27, 21, 24 . Average = 22·8 colonies.

Control-plates of No. 1 must be

applied to No. 2, viz., 4·5 colonies

60 litres of air yielded $\therefore 18\cdot3 \times 10 = 183$ „

10 „ „ 81 „

XI. *Roof of Science Schools*. July 31, 1886. Afternoon. Wind, N.W., variable in strength. Showers previous to and during experiment, also rain during previous night; the outside of the tubes was wetted by rain. 20°C .

Volume of air aspirated = 119 litres.

Construction of plug.—Glass-wool and glass-powder.

Rate.—4.8 strokes per minute.

Plug mixed with 20 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation :—

Colonies, 24, 22, 26 . . . Average = 24 colonies.

Plates similarly prepared from control-

plug gave colonies 10, 8 . Average = 9 „

119 litres of air yielded $\therefore 15 \times 20 = 300$ „

10 „ „ 25 „

XII. *Roof of Science Schools* (west side). August 4, 1886. Morning. Wind, N.W. by N. and very gentle, no rain on previous day or night. $17^{\circ}\cdot 2\text{C}$. Roads well watered.

No. 1. *Volume of air aspirated* = 60 litres.

Construction of plug.—Sugared glass-wool and sugar-powder.

Rate.—3.2 strokes per minute.

Plug mixed with 10 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation :—

Colonies, 31, 27, 24 . . . Average = 27.3 colonies.

Plates similarly prepared from control-

plug gave colonies 5, 3 . Average = 4 „

60 litres of air yielded $\therefore 23.3 \times 10 = 233$ „

10 „ „ 39 „

No. 2. *Volume of air aspirated* = 60 litres.

Construction of plug.—Sugared glass-wool and sugar-powder.

Rate.—3.4 strokes per minute.

Plug mixed with 10 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation :—

Colonies, 32, 28, 30 . . . Average = 30 colonies.

Control-plates of No. 1 must be applied

also to this.

60 litres of air yielded $\therefore 26 \times 10 = 260$ „

10 „ „ 43 „

XIII. *Roof of Science Schools* (east side). August 4, 1886. Afternoon. Wind, E. to S.E., stronger than in morning.

Volume of air aspirated = 48 litres.

Construction of plug.—Sugared glass-wool and sugar-powder.

Rate.—3·5 strokes per minute.

Plug mixed with 10 c.c. of sterilised water, 1 c.c. of which gave on plate-cultivation :—

Colonies, 103, 114 . . . Average = 108·5 colonies.

Plates similarly prepared from control-

plug gave colonies 7, 7 . Average = 7 „

48 litres of air yielded $\therefore 101\cdot5 \times 10 = 1015$ „

10 „ „ 211 „

The experiments recorded above, although yielding in many cases very concordant results, point to several imperfections in the process, of which the more important are the following :—

1. A very considerable volume of air has to be aspirated in order that the small fractions ($\frac{1}{10}$ or $\frac{1}{20}$), which can alone be examined by plate cultivation, shall yield a sufficient number of colonies for accurate estimation. Thus, even when 119 litres of air were employed, the plates (each of which represented $\frac{1}{20}$ of this volume) generally only contained from 20–30 colonies.

2. A more serious objection is to be found in the difficulty of obtaining concordant plates put up from the water or broth with which the plug is mixed. This is due to the want of homogeneity caused by the material of the plug being suspended in the liquid. It was in order to reduce this disturbing suspended matter to a minimum that I constructed the plugs of sugar-powder and glass-wool coated with sugar, so that nearly the whole plug dissolved away, this construction being also intended to ensure the detachment of the organisms from the material of the plug by which they were arrested. But even the small proportion of insoluble matter in these sugared plugs is sufficient to prevent that uniformity in the plates prepared from one and the same plug which is essential to the quantitative accuracy of the process.

That the discrepancies often observed in the case of the plates prepared with these glass-wool plugs is due to the suspended glass-wool and not to any inherent defect in the quantitative accuracy of the method of plate-cultivation in general is conclusively proved by the extraordinary concordance of the plate-cultivations of ordinary waters. Thus I may quote the following results from my examinations of the London waters for the Local Government Board :—

| | Colonies obtained from 1 c. c. of water | |
|-----------------------------------|---|-------|
| | I | II |
| Chelsea, Sept 1886 | 83 | 90 |
| West Middlesex | 31 | 23 |
| Southwark | 56 | 42 |
| Grand Junction | 19 | 16 |
| Lambeth | 61 | 57 |
| New River | 19 | 15 |
| River Thames at Hampton | 8,300 | 8,500 |
| River Lea | 3,500 | 4,000 |

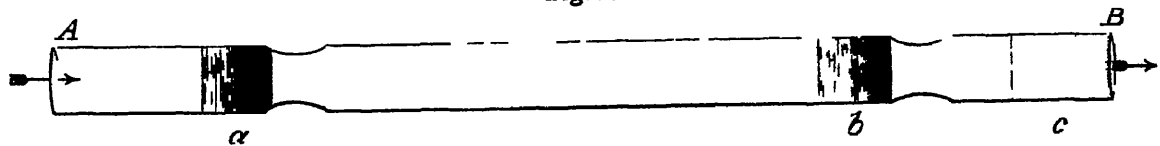
Now many of the discrepancies observed between similar plates prepared from the air plugs are largely in excess of anything observed in the case of these ordinary water plates, and it is obvious that when these discrepancies have been multiplied by 10 or by 20, as is the case, the result is still less satisfactory.

This objection applies, as already pointed out, in a still greater degree to MIQUEL'S process, since the latter is based entirely upon the *assumption* that such homogeneity is obtained in the aqueous emulsion of the plug.

In order to overcome all the difficulties to which reference has been made, I have devised the following process, which I will now describe in detail.

1. *Construction of Tubes and Plugs.*—The tubes through which the air under examination is aspirated are about 5 inches in length and $\frac{1}{4}$ inch internal diameter.

Fig. 1.



The front end (A) of the tube is open, the other extremity (B) being slightly constricted. At a distance of 1 inch from the extremity (A) the tube is constricted so as to form a support for the first plug (a), which is placed just in front of the constriction. At a distance of $2\frac{1}{2}$ inches from the plug (a) the tube is again constricted to form a support for the second plug (b), whilst resting against the constricted extremity (B) there is a third plug (c).

In constricting the tube, the extremity (B) is first constricted and the plug (c) introduced at (A), and pushed down to (B); the tube is next constricted at (b), and the second plug introduced and put in position there, whilst finally the tube is further constricted at (a), and the plug (a) introduced. Plug (a) is invariably made more pervious than (b), so that any organisms which may be carried by the current of air through (a) may find a greater resistance in (b), and thus, if (b) is found to be altogether free from organisms, it clearly shows that they must have all been arrested by (a).

In order to secure this relationship between the plugs (*a*) and (*b*), the former is constructed of a small quantity either of ordinary glass-wool or of glass-wool which has been previously coated with cane-sugar by soaking it in a saturated solution of the latter and then drying. The plug (*b*), on the other hand, is constructed of fine sugar or glass-powder (passed through sieve of 40 meshes to the linear inch), supported in front and behind by a layer of glass-wool, either plain, or coated with sugar as above. Each of these plugs is about the size of a pea, and with a little practice the packing can be easily arranged to give the requisite degree of imperviousness.

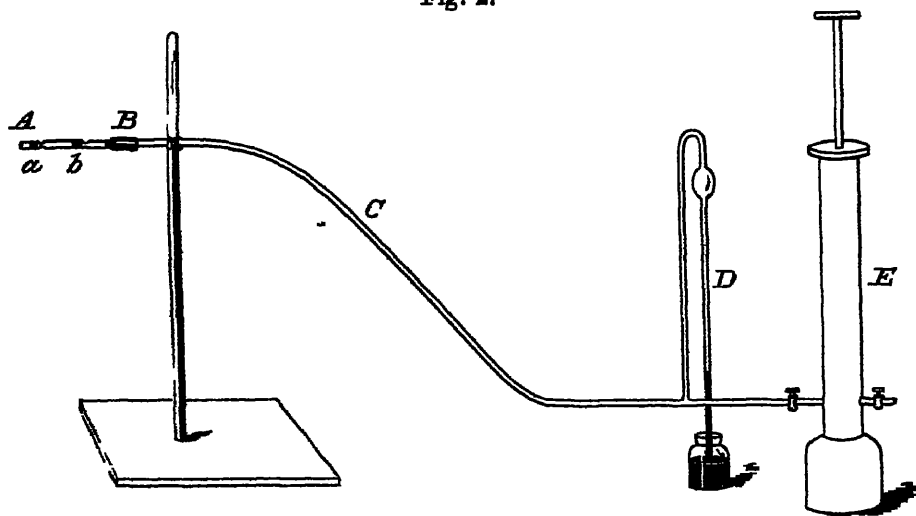
Plug (*c*) is simply a piece of cotton wool, loosely packed, and serves to protect the back of plug (*b*) from any chance contamination.

The control tubes, which are exposed during an experiment, but through which no air is aspirated, are similarly constructed, with the exception that they have no plug (*b*).

The tubes thus fitted are sterilised by being heated on 3-4 successive days, for several hours each day, to a temperature of 110° C. in the case of those containing sugar, to 160° C. in the case of those which only contain glass plugs.

A number of tubes may conveniently be sterilised at one time in a tin box, or in a piece of wide glass tubing, sealed at one end, and fitted with a plug or cork at the other. In this manner the tubes can be transported without fear of contamination to the place where the experiment is to be performed.

Fig. 2.



2. *Collection of the Sample of Air.*—The sterilised tube, prepared as above described, is carefully taken from the sterile case, and is only handled by its extremity (*B*). The latter is now attached by means of a piece of stout indiarubber tubing to a piece of lead tubing about 10 feet in length, which is clamped at this end to a retort-stand or other convenient vertical support, whilst the other extremity of the lead tube is attached to a T-piece by which it is connected, on the one hand, with an exhausting syringe, and on the other with a mercury pressure gauge, both syringe and gauge being mounted on a rigid support placed upon the ground.

By bending the lead tubing the experimental tube (AB) is brought into a horizontal position, and in out-of-door experiments the open extremity (A) is turned away at an angle from the wind. A control-tube is attached by means of wire to the vertical support, so as to rest in a precisely similar position to the experimental tube. The long piece of lead tubing enables the operator to aspirate the air by means of the hand-pump without his movements disturbing the air in the vicinity of the experimental tube, whilst, by means of a mirror placed obliquely on the ground, he is able to watch the rise and fall of the mercury in the pressure-tube. After each upward stroke of the pump, the operator waits until the pressure is equalised before making the down-stroke, and by observing this precaution each stroke of the pump corresponds to the passage through the experimental tube of a definite volume of air, which is determined once and for all by means of a gas-meter. Thus, the number of strokes measures the volume of air aspirated, whilst the number of strokes performed per minute indicates the degree of perviousness which the plugs possess. In the case of the air-pump used in the experiments recorded both above and below, 8 strokes were equivalent to $\frac{1}{14}$ th cubic foot, or 2.36 litres, 100 strokes corresponding to 30 litres.

The volume of air aspirated is varied, of course, according to the number of microbes supposed to be present, but with ordinary London air 60 strokes of the pump, or 18 litres, were found to be convenient.

In order to make what is practically a duplicate experiment, it is my general practice to alternate the aspiration of air through two different experimental tubes, taking 20 strokes through one, then 20 through the other, and so on; between the aspirations the tubes are kept in their sterile case. When the desired volume of air has been aspirated through the tube or tubes the latter are at once replaced in the sterile case, and the further treatment of the plugs proceeded with as below described.

3. *Further Treatment of the Plugs: Flask Cultivation.*—The ingenious modification of the method of plate cultivation recently introduced by my friend Dr. ESMARCH, of Berlin ('Zeitschr. f. Hygiene,' 1886, p. 293), in which the gelatine, instead of being poured out upon a glass plate, is spread over the interior surface of the test-tube, and there congealed, appeared to offer particular advantages for the treatment of the air-plugs in question. I found, however, that test-tubes were not suitable for the purpose, as the gelatine could not be violently agitated with the plug so as to disintegrate the latter without causing an inconvenient amount of froth, which, on solidification, would seriously obscure the gelatine film. In order to obviate this, and to obtain a much larger surface of gelatine, I employ flasks, 300–400 c.c.* capacity, and containing about 12 c.c. of gelatine-peptone. These flasks, with their contents, are sterilised in the ordinary way by intermittent steaming for three days.

The gelatine in these flasks having been melted at a temperature of 30° C., the

* If a very large volume of air has been aspirated, or if the air under examination is particularly rich in micro-organisms, flasks of greater capacity may be advantageously employed.

tube through which air has been aspirated is withdrawn from the sterile case, care being taken only to handle it by the extremity (B), (fig. 1). A scratch is made with a file intermediate between the plugs (*a*) and (*b*), and the tube carefully broken across. The second half, containing plugs (*b*) and (*c*), is carefully laid aside on a sterile support, whilst the first half is held between the thumb and first finger at the constriction, and the broken edge passed two or three times through a Bunsen flame, care being taken that the heat does not reach the plug. The cotton-wool stopper of one of the flasks is now withdrawn, and the extremity (A) of the tube is held vertically over the open flask, whilst the plug is carefully pushed down by means of a strong piece of sterilised copper wire, introduced from behind through the broken end of the tube. The plug falls into the flask, and the cotton-wool stopper is replaced. The second half of the tube is now taken in hand, and the broken extremity carefully passed several times through the flame. The plug (*c*), at the extremity (B), is now withdrawn by means of a hooked wire, and this end also passed through the flame. The plug (*b*) is then transferred to a second flask, in the same manner as has been described for (*a*).

The plug of the control-tube is similarly transferred to a third flask, the cotton-wool plug at the back end having been previously hooked out by means of a wire, and the broken end passed through the flame.

The gelatine in each flask is then agitated with the plug by means of a rotatory movement, which, whilst rapidly and completely disintegrating the plug, does not cause the gelatine to froth. In a few minutes the plug is quite broken up, in the case of the sugar plugs everything but the glass-wool skeleton passing into solution. The flask is now held almost horizontally under a stream of water, and by uniformly rotating it an almost perfectly even film of gelatine is spread over its inner surface. The successful spreading of the gelatine requires a little practice, and it is necessary that the stream of water should be sufficiently cold, otherwise the gelatine forms lumps; on this account, in summer iced water has generally to be used.

It is best to allow the flasks thus coated internally to remain for an hour or so in a cool place. They are then placed under a bell-jar, the internal air of which is kept saturated with moisture by means of blotting-paper soaked in water. The flasks are allowed to incubate at a temperature of about 22° C. for a period of 4-5 days. The development of the colonies takes place rather more slowly than in Hesse's tubes, which is, doubtless, due to the fact that the organisms, not being quite on the surface of the gelatine, must first grow up to the surface before a colony of any considerable dimensions can be formed. The accompanying photographs * illustrate the appearance of these flasks after proper incubation. The counting of the colonies is effected with great ease by dividing the flask with ink into segments, and holding them up against the light.

* I am indebted to Mr. Dawson for his kindness in executing these photographs for me.

Fig. 3.



EXPERIMENTAL RESULTS OBTAINED BY FLASK METHOD.

In the first instance a number of experiments were made with tubes containing only a single plug, the construction of which was varied for purposes of comparison.

Single Plug Experiments.

I. *Laboratory window* (same as above). September 18, 1886. Wind S.E., strong, variable in strength and direction. No recent rain. Roads watered in street below. 15°·1 C.

No. 1. *Time*.—11.40 A.M. to 12.10 P.M.

Volume of air = 30 litres.

Construction of plug.—Sugared glass-wool and sugar-powder.

Rate.—3·6 strokes per minute.

Colonies in flask, 131 { 89 moulds.
0 liq.*

Control-tube containing plug of sugared glass-wool.—Colonies in flask, 0.

No. 2. *Time*.—12.26 P.M. to 1.3 P.M.

Volume of air = 30 litres.

Construction of plug.—Sugared glass-wool and sugar-powder.

* liq. = Colonies causing liquefaction of the gelatine.

Rate.—3·2 strokes per minute.

Colonies in flask, 130 $\left\{ \begin{array}{l} 112 \text{ moulds.} \\ 0 \text{ liq.} \end{array} \right.$

Control-tube containing plug of sugared glass-wool.—Colonies in flask, 1.

10 litres of air yielded, therefore, in $\left\{ \begin{array}{l} \text{No. 1, 44 colonies.} \\ \text{No. 2, 48} \quad \text{,,} \end{array} \right.$

II. *Roof of Science Schools.* September 21, 1886. Wind N.E., very strong, variable in strength and direction. No recent rain. Roads watered.

No. 1. *Time.*—1.50 P.M. to 2.25 P.M. 17°·5 C.

Volume of air = 30 litres.

Construction of plug.—Sugared glass-wool and sugar-powder.

Rate.—2·9 strokes per minute.

Colonies in flask, 125 $\left\{ \begin{array}{l} 81 \text{ moulds.} \\ 0 \text{ liq.} \end{array} \right.$

Control-tube containing plug of sugared glass-wool and sugar-powder.—

Colonies in flask, 5 $\left\{ \begin{array}{l} 2 \text{ moulds.} \\ 0 \text{ liq.} \end{array} \right.$

No. 2. *Time.*—2.35 P.M. to 2.57 P.M. 16°·5 C.

Volume of air = 30 litres.

Construction of plug.—Sugared glass-wool only.

Rate.—4·9 strokes per minute.

Colonies in flask, 75 $\left\{ \begin{array}{l} 54 \text{ moulds.} \\ 0 \text{ liq.} \end{array} \right.$

No control-tube.

No. 3. *Time.*—3.0 P.M. to 3.45 P.M. 14°·5 C.

Volume of air = 30 litres.

Construction of plug.—Sugared glass-wool and sugar-powder.

Rate.—2·5 strokes per minute.

Colonies in flask, 79 $\left\{ \begin{array}{l} 49 \text{ moulds.} \\ 1 \text{ liq.} \end{array} \right.$

No control-tube.

10 litres of air yielded, therefore, in $\left\{ \begin{array}{l} \text{No. 1, 40 colonies.} \\ \text{No. 2, 25} \quad \text{,,} \\ \text{No. 3, 26} \quad \text{,,} \end{array} \right.$

III. *Roof of Science Schools.* September 22, 1886. Wind N.E., strong and variable. No recent rain. Roads watered.

No. 1. *Time.*—11.32 A.M. to 12.6 P.M.

Volume of air = 30 litres.

Construction of Plug.—Sugared glass-wool and sugar-powder.

Rate.—3.1 strokes per minute.

Colonies in flask, 81 { 40 moulds.
1 liq.

Control-tube containing plug of sugared glass-wool and sugar-powder.—

Colonies in flask, 5 { 2 moulds.
0 liq.

No. 2. *Time*.—12.17 to 12.48 P.M.

Volume of air = 30 litres.

Construction of plug.—Sugared glass-wool and sugar-powder.

Rate.—3.3 strokes per minute.

Colonies in flask, 64 { 34 moulds.
0 liq.

No control-tube, the above being considered applicable.

No. 3. *Time*.—2.42 to 4.20 P.M.

Volume of air = 60 litres.

Construction of plug.—Sugared glass-wool and sugar-powder.

Rate.—2.4 strokes per minute.

Colonies in flask, 139 { 92 moulds.
0 liq.

Control-tube containing sugared glass-wool and sugar-powder.—

Colonies in flask, 17 { 5 moulds.
0 liq.

No. 4. *Time*.—4.25 to 5 P.M.

Volume of air = 30 litres.

Construction of plug.—Sugared glass-wool and sugar-powder.

Rate.—3.4 strokes per minute.

Colonies in flask, 89 { 42 moulds.
0 liq.

No control-tube, but half the colonies found in control to No. 3 considered applicable, viz., 8.

| | | | |
|---|---|-------------------|--------------|
| 10 litres of air yielded, therefore, in | { | No. 1 (morning) | 25 colonies. |
| | | No. 2 „ | 20 „ |
| | | No. 3 (afternoon) | 20 „ |
| | | No. 4 „ | 27 „ |

IV. *Laboratory window*. October 4, 1886. No. 1 and No. 2 alternated in aspiration. 2.17 P.M. to 3.17 P.M. Wind S.E., very gentle, constant in direction.
No recent rain. 25° 6 C.

No. 1. *Volume of air* = 24 litres.

Construction of plug.—Sugared glass-wool only.

Rate.—5 strokes per minute. (Tube was exposed 18 minutes)

Colonies in flask, 139 $\left\{ \begin{array}{l} 67 \text{ moulds.} \\ 0 \text{ liq.} \end{array} \right.$

No. 2. *Volume of air* = 24 litres.

Construction of plug.—Glass-wool only.

Rate.—6.6 strokes per minute. (Tube exposed 12 minutes.)

Colonies in flask, 164 $\left\{ \begin{array}{l} 68 \text{ moulds.} \\ 0 \text{ liq.} \end{array} \right.$

Control-tube to Nos. 1 and 2 contained plug of sugared glass-wool only; it was exposed 57 minutes, but, as the flask contained only one colony, nothing need be subtracted from the results of Nos. 1 and 2, as they were only exposed 18 and 12 minutes respectively.

Nos. 3 and 4 were alternated in aspiration 3.34 P.M. to 4.7 P.M.

No. 3. *Volume of air* = 18 litres.

Construction of plug.—Sugared glass-wool only.

Rate.—4.3 strokes per minute. (Tube exposed 14 minutes.)

Colonies in flask, 114 $\left\{ \begin{array}{l} 72 \text{ moulds.} \\ 0 \text{ liq.} \end{array} \right.$

No. 4. *Volume of air* = 18 litres.

Construction of plug.—Glass-wool only.

Rate.—6.6 strokes per minute. (Tube exposed 11.5 minutes.)

Colonies in flask, 117 $\left\{ \begin{array}{l} 59 \text{ moulds.} \\ 2 \text{ liq.} \end{array} \right.$

Control-tube for Nos. 3 and 4 with sugared glass-wool plug gave colonies in flask 0.

10 litres of air yielded, therefore, in $\left\{ \begin{array}{l} \text{No. 1, 58 colonies.} \\ \text{No. 2, 68} \quad \text{,,} \\ \text{No. 3, 63} \quad \text{,,} \\ \text{No. 4, 65} \quad \text{,,} \end{array} \right.$

The above experiments tend to show that the plugs employed arrested the whole of the organisms, since in comparative experiments the more impervious plugs did not yield a larger number of colonies on cultivation than the more pervious ones. It was, however, deemed advisable in further experiments to have in each case a guarantee that all organisms were stopped, and for this purpose the doubly-plugged tubes, which have been already described, were constructed and invariably used in my later experiments.

EXPERIMENTS WITH DOUBLY PLUGGED TUBES.

I. *Roof of Science Schools.* September 23, 1886. Wind N. by N.W., fairly strong, variable in direction. No recent rain. Nos. 1 and 2 were alternated in aspiration. 2.48 P.M. to 4.56 P.M.

No. 1. *Volume of air* = 30 litres.

Construction of plugs.—(a) Sugared glass-wool only.

(b) Sugared glass-wool and sugar-powder.

Rate.—2.5 strokes per minute. (Tube exposed 47 minutes.)

Colonies in flask.—(a) $94 \begin{cases} 58 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

(b) 0.

No. 2. *Volume of air* = 27 litres.

Construction of plugs.—(a) Sugared glass-wool only.

(b) Sugared glass-wool and sugar-powder.

Rate.—1.4 stroke per minute. (Tube exposed 66 minutes.)

Colonies in flask.—(a) $100 \begin{cases} 36 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

(b) 0.

Control-tube, containing plug of sugared glass-wool only, was exposed during 81 minutes :—

Colonies in flask, $12 \begin{cases} 0 \text{ mould.} \\ 1 \text{ liq.} \end{cases}$

This corresponds to 1 organism falling on the plug in 7 minutes; from the result of No. 1, $47 \div 7 = 7$ must be subtracted, and, from No. 2, $66 \div 7 = 9$ colonies, since tube No. 1 was exposed 47 and No. 2 66 minutes.

10 litres of air yielded, therefore, in $\begin{cases} \text{No. 1, 29 colonies.} \\ \text{No. 2, 34} \end{cases}$ „

II. *Roof of Science Schools.* September 28, 1886. Wind S.W. by W., variable in direction, gentle. Dust blowing about in street below. 17° – 16° C. Nos. 1 and 2 alternated in aspiration. 3.40 P.M. to 4.58 P.M.

No. 1. *Volume of air* = 24 litres.

Construction of plugs.—(a) Sugared glass-wool only.

(b) Sugared glass-wool and sugar powder.

Rate.—2.8 strokes per minute. (Tube exposed 29 minutes.)

Colonies in flask.—(a) $63 \begin{cases} 47 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

(b) Lost.

No. 2. *Volume of air* = 24 litres.

Construction of plugs.—(a) Sugared glass-wool only.

(b) Sugared glass-wool and sugar-powder.

Rate.—2·3 strokes per minute. (Tube exposed 32 minutes.)

Colonies in flask.—(a) 65 $\begin{cases} 57 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

(b) 3 (all moulds).

Control-tube, containing plug of sugared glass-wool and sugar-powder, exposed 78 minutes.

Colonies in flask, 5 $\begin{cases} 3 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

This corresponds to 1 organism gaining access to plug in 16 minutes; 2 colonies may, therefore, be subtracted from the colonies obtained both in No. 1 and No. 2; thus

10 litres of air yielded in $\begin{cases} \text{No. 1, 25 colonies.} \\ \text{No. 2, 28} \end{cases}$ „

III. *Laboratory window.* October 5, 1886. Wind S. by S.W., fairly strong; very variable in strength and direction. No recent rain. 3.26 P.M. to 5.11 P.M. 23° C.

Comparison between glass-wool and sugared glass-wool plugs.

Nos. 1 and 2 alternated in aspiration.

No. 1. *Volume of air* = 15 litres.

Construction of plugs.—(a) *Sugared glass-wool only.*

(b) Sugared glass-wool and sugar-powder.

Rate.—1·3 strokes per minute. (Tube exposed 40 minutes.)

Colonies in flasks.—(a) 58 $\begin{cases} 50 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

(b) 0.

No. 2. *Volume of air* = 15 litres.

Construction of plugs.—(a) *Glass-wool only.*

(b) Sugared glass-wool and sugar-powder.

Rate.—1·2 strokes per minute. (Tube exposed 44 minutes.)

Colonies in flasks.—(a) 55 $\begin{cases} 50 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

(b) 0.

Control-tube containing plug of sugared glass-wool only was exposed during 50 minutes:—

Colonies in flask, 4 $\begin{cases} 0 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

This corresponds to 1 organism gaining access to plug in 12·5 minutes;

3 colonies must therefore be subtracted from No. 1 and 4 from 2.

Thus

10 litres of air yielded, therefore, in $\begin{cases} \text{No. 1 (sugar-wool), 37 colonies.} \\ \text{No. 2 (glass-wool), 34 } \end{cases}$.,

IV. *Roof of Science Schools.* October 8, 1886. Wind S.W., *very* gentle. Roads wet, pavement dry. 4.46 P.M. to 5.34 P.M. 15°·6 C.

Comparison between glass and sugar plugs.

No. 1. *Volume of air* = 18 litres.

Construction of plugs.—(a) *Glass wool only.*

(b) *Sugared glass-wool and sugar-powder.*

Rate.—5·7 strokes per minute. (Tube exposed 11 minutes.)

Colonies in flasks.—(a) 25 $\begin{cases} 22 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

(b) 0.

No. 2. *Volume of air* = 21 litres.

Construction of plugs.—(a) *Sugared glass-wool only.*

(b) *Sugared glass-wool and sugar-powder.*

Rate.—5·7 strokes per minute. (Tube exposed 15 minutes.)

Colonies in flasks.—(a) 22 $\begin{cases} 16 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

(b) 0.

Control-tube, containing plug of sugared glass-wool only, exposed 53 minutes; flask contained 0 colonies.

10 litres of air yielded, therefore, in $\begin{cases} \text{No. 1 (glass-wool), 14 colonies.} \\ \text{No. 2 (sugar-wool), 10 } \end{cases}$.,

V. *North Chemical Laboratory.* October 9, 1886. After departure of students. Empty. 5.26 P.M. to 6.8 P.M. Nos. 1 and 2 alternated in aspiration

No. 1. *Volume of air* = 18 litres.

Construction of plugs.—(a) *Glass-wool only.*

(b) *Sugared glass-wool and sugar-powder.*

Rate.—5·7 strokes per minute. (Tube exposed 12 minutes.)

Colonies in flasks.—(a) 53 $\begin{cases} 48 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

(b) 3 $\begin{cases} 2 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

No. 2. *Volume of air* = 18 litres.

Construction of plugs.—(a) *Sugared glass-wool.*

(b) *Sugared glass-wool and sugar-powder.*

Rate.—5·7 strokes per minute. (Tube exposed 12 minutes.)

Colonies in flasks.—(a) $69 \begin{cases} 59 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$
 (b) 0.

Control-tube, containing plug of sugared glass-wool only, exposed 44 minutes; flask contained 0 colonies.

10 litres of air yielded, therefore, in $\begin{cases} \text{No. 1 (glass-wool), 31 colonies.} \\ \text{No. 2 (sugar-wool), 38 } \end{cases}$ „

VI. *Roof of Science Schools*. October 11, 1886. Wind S.W., very gentle. Roads wet, pavement dry. 13°·3 C. Comparison between two sugar plugs, one of which had become partially converted into caramel in sterilisation; the two tubes were alternated in aspiration. 4.34 P.M. to 5.21 P.M.

No. 1. *Volume of air* = 21 litres.

Construction of plugs.—(a) Sugared glass-wool (not charred).

(b) Sugared glass-wool and sugar-powder.

Rate.—6·6 strokes per minute. (Tube exposed 13 minutes.)

Colonies in flasks.—(a) $32 \begin{cases} 25 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$
 (b) $3 \begin{cases} 2 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

No. 2. *Volume of air* = 21 litres.

Construction of plugs.—(a) Sugared glass-wool (partly charred).

(b) Sugared glass-wool and sugar-powder.

Rate.—4 strokes per minute. (Tube exposed 19 minutes.)

Colonies in flasks.—(a) $39 \begin{cases} 24 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$
 (b) 0.

Control-tube, containing plug of sugared glass-wool only, exposed 51 minutes:—

Colonies in flask, $17 \begin{cases} 16 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

This corresponds to 1 organism in 3 minutes gaining access to the plugs; 4 colonies must, therefore, be subtracted from No. 1, and 6 colonies from No. 2. Thus

10 litres of air yielded, therefore, in $\begin{cases} \text{No. 1, 15 colonies.} \\ \text{No. 2, 16 } \end{cases}$ „

VII. *Roof of Science Schools.* October 13, 1886. Wind W. by S.W., strong, but constant. Much rain during day and night previous. 10°·6 C. 4.47 P.M. to 5.30 P.M. Comparison between glass and sugar plugs. Tubes alternated in aspiration.

No. 1. *Volume of air* = 24 litres.

Construction of plugs.—(a) *Sugared glass-wool.*

(b) *Sugared glass-wool and sugar-powder.*

Rate.—5 strokes per minute. (Tube exposed 17 minutes.)

Colonies in flasks.—(a) 38 { 22 moulds.
0 liq.
(b) 0.

No. 2. *Volume of air* = 27 litres.

Construction of plugs.—(a) *Glass-wool only.*

(b) *Sugared glass-wool and sugar-powder.*

Rate.—7·2 strokes per minute. (Tube exposed 14 minutes.)

Colonies in flasks.—(a) 61 { 39 moulds.
1 liq.
(b) 0.

Control-tube, containing sugared glass-wool plug, exposed 46 minutes:—
Colonies in flask, 0.

10 litres of air yielded, therefore, in { No. 1 (sugar-wool), 16 colonies.
No. 2 (glass-wool), 23 „

VIII. *Roof of Science Schools.* October 14, 1886. Wind W. by S.W. Moderate. Constant in direction. Roads wet, pavement dry. 4.8 P.M. to 4.56 P.M. 12°·2 C. Comparison between glass and sugar plugs. Tubes alternated in aspiration.

No. 1. *Volume of air* = 24 litres.

Construction of plugs.—(a) *Glass-wool only.*

(b) *Sugared glass-wool and sugar-powder.*

Rate.—6·6 strokes per minute. (Tube exposed 13 minutes.)

Colonies in flasks.—(a) 44 { 23 moulds.
0 liq.
(b) 3 { 1 mould.
0 liq.

No. 2. *Volume of air* = 24 litres.

Construction of plugs.—(a) *Sugared glass-wool.*

(b) *Sugared glass-wool and sugar-powder.*

Rate.—5 strokes per minute. (Tube exposed 16 minutes.)

Colonies in flasks.—(a) 62 $\left\{ \begin{array}{l} 47 \text{ moulds.} \\ 1 \text{ liq.} \end{array} \right.$
 (b) 0.

Control-tube, containing plug of sugared glass-wool, exposed 50 minutes.
 Colonies in flask, 0.

10 litres of air yielded, therefore, in $\left\{ \begin{array}{l} \text{No. 1 (glass-wool), 18 colonies.} \\ \text{No. 2 (sugar-wool), 26 } \text{,,} \end{array} \right.$

The experiments recorded above show that when doubly plugged tubes are employed the organisms are almost invariably arrested by the first plug, and that the second or (b) plug only occasionally yields any, and then very few, colonies, although in all cases the second plug was constructed of a far more impervious material than the first plug, by introducing powder in addition to wool into its composition.

The results obtained with the control-tubes, which were in each case simultaneously exposed, show that the number of organisms gaining access to the tubes, irrespectively of aspiration, is, in general, *nil*, or at most forms a very small fraction of the number collected by aspiration in the same time. The exposure of these control-tubes involves very little additional trouble, and should be invariably resorted to, and if any organisms are found in them a simple correction can be made, as has been done in the above experiments.

As regards the most advantageous construction of the front or (a) plugs, it would appear that there is nothing to choose between simple glass-wool and sugared glass-wool as regards their power of arresting the organisms, whilst the sugared wool leaves the gelatine-film in the flask rather clearer; but this slight difference is of no material consequence.

Comparison of Flask-Method with HESSE's Method.

Having found that the results obtained by means of the new flask-method were very concordant *inter se*, it became of interest to institute a comparison between the results yielded by this method and those by HESSE's apparatus under precisely similar conditions.

There is an interest attaching to this comparison entirely apart from the question of which of the two processes is most reliable in the results which it yields, or of which is most advantageous for practical purposes, for by a comparison of the results obtained by the two methods it is possible to ascertain whether the living organisms suspended in air are in an isolated state, or whether they are present in aggregated masses. HESSE's researches show that the suspended organisms of the air are specifically isolated, that is to say, that organisms of two or more different kinds are not found adhering together, for the individual colonies which result from their deposition in his tubes, or on the surface of any solid medium, are pure cultivations and not mixtures of organisms.

My experience is entirely in accord with HESSE's observations on this point. It is obvious, however, that any individual colony in HESSE's tubes may result either from the deposition of a single organism or from the deposition of an aggregate of organisms of one and the same kind. Now, in the new method described above, any aggregates of this kind would, in the process of agitating the plug with the gelatine, become broken up, and a number of colonies would result from that which, in HESSE's method, would yield a single colony only. In comparing the two methods, there was, therefore, no *a priori* reason for anticipating that the results obtained should bear any similarity to each other, and, since there is nothing to render the existence of such aggregates improbable, it would not have been at all surprising to find the number of colonies yielded by the flask-method in excess, and even very largely in excess, of the number obtained by HESSE's apparatus from the same volume of the same air.

A number of experiments were carried out with this double object in view, the results of which are recorded below.

I. *Roof of Science Schools.* September 24, 1886. Wind N.W., very gentle, but variable in strength and direction. No recent rain. Roads watered, pavement dry. 12°·8 C.

No. 1. *Time.*—3.1 to 3.20 P.M.

Flask-method.—(Single plug.)

Volume of air = 12 litres.

Construction of plug.—Sugared glass-wool and sugar-powder.

Rate.—2·2 strokes per minute. (Tube exposed 19 minutes.)

Colonies in flask, 37 { 15 moulds.
1 liq.

Control tube, containing plug of sugared glass-wool and sugar-powder, exposed the whole time occupied by No. 1.

Colonies in flask, 0.

No. 2. HESSE's method.

Time.—3.30 P.M. to 4.20 P.M.

Volume of air = 12 litres.

Tube contained 71 colonies { 21 moulds.
2 liq.

Control-tube contained 36 colonies { 7 moulds.
1 liq.

II. *Roof of Science Schools.* September 25, 1886. Wind S.W., very gentle. No recent rain, roads watered, pavement dry, 12°·8 C.

No. 1. *Time.*—5.35 P.M. to 6.0 P.M. *Flask method* (single plug).

Volume of air = 18 litres.

Construction of plug.—Sugared glass-wool and sugar-powder.

Rate.—2.5 strokes per minute.

Colonies in flask, 39 $\left\{ \begin{array}{l} 25 \text{ moulds.} \\ 1 \text{ liq.} \end{array} \right.$

Control tube, containing plug of sugared glass-wool and sugar-powder, exposed the whole time occupied by No. 1.

Colonies in flask, 0.

12 litres of air yielded, therefore, 22 colonies.

No. 2. *HESSE's method.*

Time.—6.20 P.M. to 7.15 P.M.

Volume of air = 12 litres.

Tube contained 32 colonies $\left\{ \begin{array}{l} 19 \text{ moulds.} \\ 1 \text{ liq.} \end{array} \right.$

Control-tube contained 16 colonies $\left\{ \begin{array}{l} 5 \text{ moulds.} \\ 1 \text{ liq.} \end{array} \right.$

III. *Chemical Laboratory*. October 15, 1886. Shortly after students had left.
4.50 P.M. to 5.32 P.M. 16°.9 C.

Nos. 1 and 2. *Flask method*.—(Double plugs.)

No. 3. *HESSE's method.*

Nos. 1 and 2 were alternated in aspiration. No. 3 simultaneously aspirated in close proximity, the tubes being inclined at the same angle.

No. 1. *Volume of air* = 24 litres.

Construction of plugs.—(a) Glass-wool only.

(b) Sugared glass-wool and sugar-powder.

Rate.—8 strokes per minute. (Tube exposed 12 minutes.)

Colonies in flasks.—(a) 73 $\left\{ \begin{array}{l} 40 \text{ moulds.} \\ 1 \text{ liq.} \end{array} \right.$

(b) 1 mould.

No. 2. *Volume of air* = 18 litres

Construction of plugs.—(a) Sugared glass-wool.

(b) Sugared glass-wool and sugar-powder.

Rate.—7.3 strokes per minute. (Tube exposed 9 minutes.)

Colonies in flasks.—(a) 43 $\left\{ \begin{array}{l} 23 \text{ moulds.} \\ 0 \text{ liq.} \end{array} \right.$

(b) 2 moulds.

Control-tube, containing plug of sugared glass-wool only, exposed 46 minutes.

Colonies in flask, 0.

10 litres of air yielded, therefore, in $\left\{ \begin{array}{l} \text{No. 1 (glass-wool), 31 colonies.} \\ \text{No. 2 (sugar-wool), 25 „} \end{array} \right.$

No. 3. HESSE's *method*.*Time*.—4.50 P.M. to 5.35 P.M.*Volume of air* = 10 litres.

Colonies in tube . . . 30 $\left\{ \begin{array}{l} 16 \text{ moulds.} \\ 1 \text{ liq.} \end{array} \right.$

„ *control-tube* . 5 $\left\{ \begin{array}{l} 2 \text{ moulds.} \\ 0 \text{ liq.} \end{array} \right.$

IV. *North Chemical Laboratory*. October 16, 1886. Saturday afternoon; quite empty, and only a few (12) students had been working in the morning. 2.3 P.M. to 2.35 P.M. 16°·9 C.

Nos. 1 and 2. *Flask method*.—(Double plugs.)No. 3. HESSE's *method*.

Nos. 1 and 2 were alternated in aspiration; No. 3 was simultaneously aspirated in close proximity, the tubes being inclined at the same angle.

No. 1. *Volume of air* = 18 litres.*Construction of plugs*.—(a) Glass-wool only.

(b) Sugared glass-wool and sugar-powder.

Rate.—5·3 strokes per minute. (Tube exposed 14 minutes.)

Colonies in flasks.—(a) 35 $\left\{ \begin{array}{l} 22 \text{ moulds.} \\ 0 \text{ liq.} \end{array} \right.$

(b) 1 mould.

No. 2. *Volume of air* = 12 litres.*Construction of plugs*.—(a) Sugared glass-wool.

(b) Sugared glass-wool and sugar-powder.

Rate.—5 strokes per minute. (Tube exposed 8 minutes.)

Colonies in flasks.—(a) 26 $\left\{ \begin{array}{l} 14 \text{ moulds.} \\ 0 \text{ liq.} \end{array} \right.$

(b) 0.

Control-tube, containing plug of sugared glass-wool, exposed 37 minutes:—

Colonies in flask, 0.

9 litres of air yielded, therefore, in $\left\{ \begin{array}{l} \text{No. 1 (glass-wool), 18 colonies.} \\ \text{No. 2 (sugar-wool), 20 } \end{array} \right.$ „

No. 3. HESSE's *method*.*Volume of air* = 9 litres.

Colonies in tube . . . 13* $\left\{ \begin{array}{l} 11 \text{ moulds.} \\ 1 \text{ liq.} \end{array} \right.$

„ *control-tube* . 2 $\left\{ \begin{array}{l} 1 \text{ mould.} \\ 1 \text{ liq.} \end{array} \right.$

* A liquefying colony had spread considerably, and may therefore have obliterated some of the others, and the result is, therefore, probably somewhat too low.

V. *Roof of Science Schools.* October 19, 1886. Wind S.E., gentle, constant in direction. Rain during morning, roads wet, roof and pavement nearly dry.

4.58 P.M. to 5.32 P.M.

Nos. 1 and 2. *Flask method.*—(Double plugs.)

No. 3. *HESSE's method.*

Nos. 1 and 2 alternated in aspiration. No. 3 aspirated simultaneously in close proximity, and with the tubes inclined at the same angle.

No. 1. *Flask method.*

Volume of air = 18 litres.

Construction of plugs.—(a) Glass-wool only.

(b) Sugared glass-wool and sugar-powder.

Rate.—8 strokes per minute. (Tube exposed 9 minutes.)

Colonies in flasks.—(a) 53 $\left\{ \begin{array}{l} 42 \text{ moulds.} \\ 0 \text{ liq.} \end{array} \right.$

(b) 0.

No. 2. *Flask method.*

Volume of air = 18 litres.

Construction of plugs.—(a) Sugared glass-wool.

(b) Sugared glass-wool and sugar-powder.

Rate.—5 strokes per minute. (Tube exposed 13 minutes.)

Colonies in flasks.—(a) 56 $\left\{ \begin{array}{l} 24 \text{ moulds.} \\ 0 \text{ liq.} \end{array} \right.$

(b) 0.

Control-tube, containing plug of sugared glass-wool, exposed 36 minutes:—

Colonies in flask, 0.

9 litres of air yielded, therefore, in $\left\{ \begin{array}{l} \text{No. 1 (glass-wool), 27 colonies.} \\ \text{No. 2 (sugar-wool), 28 } \text{,,} \end{array} \right.$

No. 3. *HESSE's method.*

Volume of air = 9 litres.

Colonies in tube . . . 31 $\left\{ \begin{array}{l} 16 \text{ moulds.} \\ 0 \text{ liq.} \end{array} \right.$

„ *control-tube* . 2* moulds.

VI. *Roof of Science Schools.* October 20, 1886. Wind W. by S.W., gentle, but increasing during experiment; roads wet, pavement dry. Rain during previous night. 12°·8 C.

Nos. 1 and 2. *Flask method.*—(Double plugs.)

No. 3. *HESSE's method.*

Nos. 1 and 2 alternated in aspiration; No. 3 aspirated simultaneously in close proximity, the tubes being inclined at the same angle.

* This tube was somewhat overgrown by a liquefying colony, so that this result is probably too low.

No. 1. *Flask method.**Volume of air* = 24 litres.*Construction of plugs.*—(a) Glass-wool only.

(b) Sugared glass-wool and sugar-powder.

Rate.—8 strokes per minute. (Tube exposed 11 minutes.)*Colonies in flask.*—(a) 45 $\begin{cases} 36 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

(b) 0.

No. 2. *Flask method.**Volume of air* = 18 litres.*Construction of plugs.*—(a) Sugared glass-wool.

(b) Sugared glass-wool and sugar-powder.

Rate.—6.6 strokes per minute. (Tube exposed 11 minutes.)*Colonies in flasks.*—(a) 26 $\begin{cases} 19 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

(b) 0.

Control-tube, containing plug of sugared glass-wool, exposed 43 minutes:—

Colonies in flask, 1 mould.

10 litres of air yielded, therefore, in $\begin{cases} \text{No. 1 (glass-wool), 19 colonies.} \\ \text{No. 2 (sugar-wool), 14 } \end{cases}$ „No. 3. *HESSE'S method.**Volume of air* = 10 litres.*Colonies in tube*, 26 $\begin{cases} 20 \text{ moulds.} \\ 1 \text{ liq.} \end{cases}$ „ *control-tube.* Lost through spread of liquefying colony, but several other colonies were visible.

VII. *Roof of Science Schools.* October 22, 1886. Wind S.W., changing to W.; very gentle, but variable. Roads wet, roof still wet in places from heavy dew. Foggy morning, which had cleared to sunshine. 9°.4 C. 10.44 A.M. to 11.25 A.M.

Nos. 1 and 2. *Flask method.*—(Double plugs.)No. 3. *HESSE'S method.*

Nos. 1 and 2 were alternated in aspiration. No. 3 was aspirated simultaneously in close proximity, the tubes being inclined at the same angle.

No. 1. *Flask method.**Volume of air* = 18 litres.*Construction of plugs.*—(a) Glass-wool only.

(b) Sugared glass-wool and sugar-powder.

Rate.—3·6 strokes per minute. (Tube exposed 18 minutes.)

Colonies in flasks.—(a) 17 $\begin{cases} 9 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

(b) 0.

No. 2. *Flask method*.

Volume of air = 12 litres.

Construction of plugs.—(a) Sugared glass-wool.

(b) Sugared glass-wool and sugar-powder.

Rate.—2·9 strokes per minute. (Tube exposed 14 minutes.)

Colonies in flasks.—(a) 13 $\begin{cases} 6 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

(b) 0.

11 litres of air yielded, therefore, in $\begin{cases} \text{No. 1 (glass-wool), 10 colonies.} \\ \text{No. 2 (sugar-wool), 12 } \end{cases}$ „

No. 3. *HESSE's method*.

Volume of air = 11 litres.

Colonies in tube, 18 $\begin{cases} 13 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

„ control tube, 10 $\begin{cases} 2 \text{ moulds.} \\ 1 \text{ liq.} \end{cases}$

VIII. *Roof of Science Schools*. October 25, 1886. Wind E.; very strong and gusty, but fairly constant in direction. Rain earlier in the morning. Roads wet, pavement dry, roof damp. 11°·3 C. 10.44 A.M. to 11.26 A.M.

Nos. 1 and 2. *Flask method*.—(Double plugs.)

No. 3. *HESSE's method*.

Nos. 1 and 2 were alternated in aspiration; No. 3 was aspirated simultaneously in close proximity, the tubes being inclined at the same angle.

No. 1. *Flask method*.

Volume of air = 18 litres.

Construction of plugs.—(a) Glass-wool only.

(b) Sugared glass-wool and sugar-powder.

Rate.—5 strokes per minute. (Tube exposed 15 minutes.)

Colonies in flasks.—(a) 37 $\begin{cases} 28 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

(b) Lost.

No. 2. *Flask method*.

Volume of air = 18 litres.

Construction of plugs.—(a) Sugared glass-wool.

(b) Sugared glass-wool and sugar-powder.

Rate.—5·7 strokes per minute. (Tube exposed 13 minutes.)

Colonies in flasks.—(a) 43 $\begin{cases} 20 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$
(b) 0.

Control-tube, containing plug of sugared glass-wool, exposed 43 minutes.

Colonies in flask, 1 $\begin{cases} 0 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

10 litres of air yielded, therefore, in $\begin{cases} \text{No. 1 (glass-wool), 21 colonies.} \\ \text{No. 2 (sugar-wool), 24 } \end{cases}$ „

No. 3. *HESSE's method*.

Volume of air = 10 litres.

Colonies in tube . . . 68 $\begin{cases} 25 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

„ *control-tube* 17* $\begin{cases} 3 \text{ moulds.} \\ 1 \text{ liq.} \end{cases}$

IX. *Chemical Laboratory* (private). October 27, 1886. Windows and door closed;
3 persons in room, but not moving about. 10.59 A.M. to 11.54 A.M. 16°·9 C.

Nos. 1 and 2. *Flask method*.—(Double plugs.)

No. 3. *HESSE's method*.

Nos. 1 and 2 alternated in aspiration; No. 3 aspirated simultaneously in
close proximity.

No. 1. *Flask method*.

Volume of air = 18 litres.

Construction of plugs.—(a) Sugared glass-wool.

(b) Sugared glass-wool and sugar-powder.

Rate.—5·3 strokes per minute. (Tube exposed 12 minutes.)

Colonies in flasks.—(a) 55 $\begin{cases} 40 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$
(b) 3 moulds.

No. 2. *Flask method*.

Volume of air = 18 litres.

Construction of plugs.—(a) Glass-wool only.

(b) Sugared glass-wool and sugar-powder.

Rate.—7·2 strokes per minute. (Tube exposed 10 minutes.)

Colonies in flasks.—(a) 55 $\begin{cases} 37 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$
(b) 5 $\begin{cases} 2 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

* This tube was much spoilt by liquefying colony; the number is, therefore probably too small.

Control-tube, containing plug of sugared glass-wool, exposed 41 minutes : —
Colonies in flask, 0.

10 litres of air yielded, therefore, in $\begin{cases} \text{No. 1 (sugar-wool), 32 colonies.} \\ \text{No. 2 (glass-wool), 33 } \end{cases}$ „

No. 3. *HESSE's method.*

Volume of air = 10 litres.

Colonies in tube . . . 32 $\begin{cases} 14 \text{ moulds.} \\ 1 \text{ liq.} \end{cases}$

„ *control-tube* 2 $\begin{cases} 1 \text{ mould.} \\ 0 \text{ liq.} \end{cases}$

X. *Roof of Science Schools.* October 29, 1886. Wind S.W., gentle, with occasional gusts; fairly constant in direction. Roads and pavement wet. Roof still damp from previous rain. 16°·3 C. 10.51 A.M. to 11.38 A.M.

No. 1. *Flask method.*—(Double plugs.)

No. 2. „ (Lost.)

No. 3. *HESSE's method.*

Nos. 1 and 2 were alternated in aspiration; No. 3 was simultaneously aspirated in close proximity, the tubes being inclined at the same angle.

No. 1. *Flask method.*

Volume of air = 18 litres.

Construction of plugs.—(a) Glass-wool only.

(b) Sugared glass-wool and sugar-powder.

Rate.—3·6 strokes per minute. (Tube exposed 17 minutes.)

Colonies in flasks.—(a) 30 $\begin{cases} 27 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$
(b) 0.

Control-tube, containing plug of sugared glass-wool, exposed 49 minutes :—

Colonies in flask, 1 $\begin{cases} 0 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

12 litres of air yielded, therefore, in No. 1, 20 colonies.

No. 3. *HESSE's method.*

Volume of air = 12 litres.

Colonies in tube . . . 35 $\begin{cases} 12 \text{ moulds.} \\ 1 \text{ liq.} \end{cases}$

„ *control-tube* . 6 $\begin{cases} 3 \text{ moulds.} \\ 0 \text{ liq.} \end{cases}$

These experiments clearly demonstrate that the results obtained by the flask method and by *HESSE's method* are in remarkable concord when the experiments are

performed in-doors, or on very calm days out-of-doors, whilst when performed in a disturbed atmosphere, more especially when the direction of the wind is variable, or, what amounts to the same thing, when the number of colonies in the control-tube, which I employ with HESSE's apparatus, amounts to a considerable fraction of the number found in the HESSE tube through which air has been aspirated, the results obtained by HESSE's method are markedly in excess of those obtained by the flask method.

The only conclusion with regard to HESSE's method which can be drawn from the issue of these experiments is this, that in undisturbed air it yields results which can lay claim to a fair degree of accuracy, but that when the atmosphere is disturbed, more especially by currents variable in direction, the results are often very considerably above the truth. For practical purposes, therefore, it may be taken that when a control-HESSE-tube, such as I have employed, yields practically no colonies the result by HESSE's method is accurate, but that when such a tube yields a considerable number of colonies the result is too high.

The fact that the flask method and HESSE's method yield practically coincident results when the latter (HESSE's) is employed under circumstances in which it is reliable (*i.e.*, in calm air) gives a clear and unmistakable answer to the question, which I have already referred to, as to whether the suspended organisms in the air exist there in an isolated condition, or in aggregates of greater or less magnitude.

The coincidence between the results obtained by these two methods is only explicable, in fact, on the supposition that the aerial micro-organisms are present as isolated individuals, for, were they present in aggregates, the results yielded by the flask-method would be in excess; and if the magnitude of the aggregates was great, in enormous excess of those obtained by HESSE's method, as in the process of agitation with the liquid gelatine to which they are subjected in the flask-method, these aggregates would necessarily become broken up into lesser aggregates, at any rate, if not into the ultimate individuals of which they are composed.

HESSE, in his well-known paper "Ueber quantitative Bestimmung der in der Luft enthaltenen Mikroorganismen" ('Mittheilungen a. d. Kaiserl. Gesundheitsamte,' vol. 2, 1884, p. 187), draws the following conclusion with regard to this point:—
*"The conspicuous fact that the extreme colonies formed in the tube are moulds indicates that the mould-germs present in the air are on the average lighter than the bacterial germs, and leads to the conclusion that the aerial bacteria are not present as isolated individuals, but as aggregates of individuals or adhering to carriers in such a manner that on the average they weigh heavier than the mould-germs."**

* "In beiden Röhren sind die äussersten Colonien Pilzcolonien. Diese . . . auffällig hervortretende Erscheinung zeigt, dass die in der Luft enthaltenen Pilzkeime durchschnittlich leichter sind als die Bacterienkeime, und führt zu dem Schlusse, dass die Bacterien nicht als einzelne Individuen isolirt in der Luft enthalten sind, sondern als Häufchen von Individuen, oder an Trägern haftend derart, dass sie durchschnittlich etwas schwerer wiegen als Pilzsporen."

The one alternative suggested by HESSE as possibly accounting for the undoubtedly slower deposition of the mould germs is thus rendered untenable, since neither mould germs nor bacterial germs appear to be present in air as aggregates, but as isolated individuals, and some other explanation of this difference in their behaviour must therefore be resorted to.

The advantages which appear to me to attach to the new method of quantitatively determining the micro-organisms of the air, which I have described in the preceding pages, may be briefly summarized as follows :—

1. The method possesses all the well-recognised advantages pertaining to the use of a solid cultivating medium.

2. The results, as tested by the comparison of parallel experiments, can lay claim to a high degree of quantitative accuracy.

3. The results, as tested by control experiments, are not appreciably affected by aerial currents, which prove such a disturbing factor in the results obtained by some other methods.

4. The collection of an adequate sample of air occupies a very short space of time, so that a much larger volume of air can be conveniently operated upon than is the case with HESSE's method. Thus, whilst the aspiration of 10 litres of air through HESSE's apparatus takes about three-quarters of an hour, by the new method about 48 litres can be drawn through the tube in the same time, whilst a better plan is to take two tubes and alternately draw a definite volume of air through each, as by this means duplicate results are obtained.

5. As the whole plug upon which the organisms from a given volume of air are deposited is submitted to cultivation without subdivision, no error is introduced through the multiplication of results obtained from aliquot parts, and all the great difficulties attending equal subdivision are avoided.

6. The risk of aerial contamination in the process of *flask-cultivation* is practically nil.

7. The apparatus required being very simple and highly portable, the method is admirably adapted for the performance of experiments at a distance from home and in the absence of special laboratory appliances.

I must express my thanks to MR. HART, A.R.S.M., for the zeal and patience with which he has assisted me in this investigation.

ADDENDUM.

(Added April 19, 1887.)

The following experiments, made by this new method on St. Paul's Cathedral, may be recorded as further illustrations of the accuracy of the process. The experiments were made on November 19, 1886, the air being examined on the Golden Gallery, which is at the top of the dome; on the Stone Gallery, which encircles the base of the dome; and in the Churchyard at the bottom of the Cathedral.

St. Paul's, Golden Gallery.—November 19, 1886. Wind due S., strong. Roads wet, pavement dry. 1.10 to 2.20 P.M. 9°·7 C. The air was aspirated through three tubes alternately.

No. 1. *Volume of air* = 18 litres.

Construction of plugs.—(a) Sugared glass-wool.

(b) Sugared glass-wool and sugar-powder.

Rate.—3·3 strokes per minute. (Tube exposed 19 minutes.)

Colonies in flasks.—(a) 18 { 8 moulds.
0 liq.

(b) 0.

No. 2. *Volume of air* = 21 litres.

Construction of plugs.—(a) Glass-wool.

(b) Sugared glass-wool and sugar-powder.

Rate.—5 strokes per minute. (Tube exposed 18 minutes.)

Colonies in flasks.—(a) 23 { 14 moulds.
0 liq.

(b) 0.

No. 3. *Volume of air* = 18 litres.

Construction of plugs.—(a) Glass-wool.

(b) Sugared glass-wool and sugar-powder.

Rate.—6·6 strokes per minute. (Tube exposed 10 minutes.)

Colonies in flasks.—(a) 20 { 15 moulds.
0 liq.

(b) 0.

Control-tube exposed 75 minutes.

Colonies in flask.—1 (mould).

Stone Gallery.—November 19, 1886. 2.56 P.M. to 3.52 P.M. 10°·5 C.

No. 1. *Volume of air* = 12 litres.

Construction of plugs.—(a) Sugared glass-wool.

(b) Sugared glass-wool and sugar-powder.

Rate.—3·6 strokes per minute. (Tube exposed 11·5 minutes.)

Colonies in flasks.—(a) 42 $\left\{ \begin{array}{l} 26 \text{ moulds.} \\ 0 \text{ liq.} \end{array} \right.$

(b) 0.

No. 2. *Volume of air* = 12 litres.

Construction of plugs.—(a) Glass-wool.

(b) Sugared glass-wool and sugar-powder.

Rate.—4 strokes per minute. (Tube exposed 11 minutes.)

Colonies in flasks.—(a) 48 $\left\{ \begin{array}{l} 27 \text{ moulds.} \\ 0 \text{ liq.} \end{array} \right.$

(b) 1. (0 mould, 0 liq.)

Control-tube exposed 56 minutes.

Colonies in flask.—1 (liq.).

St. Paul's Churchyard.—November 19, 1886. 4.13 P.M. to 4.25 P.M. 11°·1 to 12°·2 C.

Volume of air = 24 litres.

Construction of plugs.—(a) Glass-wool.

(b) Sugared glass-wool and sugar-powder.

Rate.—7·5 strokes per minute. (Tube exposed 11 minutes.)

Colonies in flasks.—(a) 112 $\left\{ \begin{array}{l} 66 \text{ moulds.} \\ 2 \text{ liq.} \end{array} \right.$

(b) 0.

Control-tube exposed 16 minutes.

Colonies in flask.—0.

SUMMARY.

St. Paul's Cathedral.

| | | | | | |
|----------------|---|------|------------------------------------|----|---|
| Golden Gallery | { | I. | 10 colonies from 10 litres of air. | | |
| | | II. | 11 | „ | „ |
| | | III. | 11 | „ | „ |
| Stone Gallery | { | I. | 35 | „ | „ |
| | | II. | 41 | „ | „ |
| Churchyard | . | . | . | 47 | „ |

VI. *A Minute Analysis (Experimental) of the Various Movements produced by stimulating in the Monkey different Regions of the Cortical Centre for the Upper Limb, as defined by Professor FERRIER.*

By CHARLES E. BEEVOR, M.D., M.R.C.P., and VICTOR HORSLEY, B.S., F.R.C.S.,
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Received June 4,—Read June 10, 1886.

[PLATE 7.]

THE following research was undertaken as a necessary preface to an investigation, which we are at present engaged upon, into the localisation of motor function in the cervical enlargement of the spinal cord.

Briefly speaking, the experiments from which the following conclusions have been drawn consisted in an elaborate examination of the movements elicited by stimulating with the interrupted induced current every part of the motor cortex in the Monkey, in which the upper limb is primarily represented, as first described by Professor FERRIER.

Before entering, however, on a detailed description of the present research, it will be necessary to discuss shortly the anatomical features of the parts of the cortex concerned.

Anatomy.—A glance at the accompanying diagram of the external or convex surface of the left cerebral hemisphere of the Macaque Monkey shows that FERRIER's motor region is bounded inferiorly by the fissure of SYLVIVS, posteriorly by the intra-parietal sulcus, superiorly by the margin of the hemisphere, and anteriorly by a narrow strip of grey matter* in front of the vertical limb of the præcentral sulcus, and also by that sulcus itself. The central point of this area is the middle third of the ascending frontal convolution, and in this portion of the cortex and around it we have the cortical "centre" for the movements of the opposite upper limb.

Before enumerating the functions of this portion of the cortex it is incumbent upon us to draw attention to certain minute characteristics of the various sulci of this region, these being of great constancy, and therefore of primary importance in aiding exact localisation of function.

* By this is meant portions of the bases of the frontal convolutions, the extent of which can be mapped out by electrical excitation.

Proceeding from the best known of these to those which hitherto have escaped especial notice, we will commence with the fissure of ROLANDO.

The *fissure of ROLANDO*, in all the species of Monkey on which we have experimented, runs outwards, forwards, and downwards, forming an angle of 50° to 55° with the mesial margin of the hemisphere. In its course it presents the following changes of shape, which in our experience are perfectly constant. Thus, for the uppermost quarter of its extent it presents a distinct, though slight, curve with the convexity forwards; in the next, *i.e.* the second quarter, it is slightly curved, with the convexity in the opposite direction, *viz.*, posteriorly; and this curvature, concave anteriorly, runs down into the third quarter, in which part of its course the fissure presents a well-marked bend forwards. To this bend we would now direct especial attention, for it can be demonstrated to exist perfectly distinctly in Man and most Monkeys. Further, in the *Monkeys* we have examined, this bend is situated, as we have already said, in the third quarter of the fissure; its apex or central point is rounded, and is just above the horizontal level of the lower end of the intra-parietal sulcus, while, at the same time, it is well below the level of the highest point of the præcentral sulcus. From the apex of this bend the fissure of ROLANDO in its lowest fourth slopes almost vertically downwards (vertically signifying at right angles to the longitudinal fissure) towards the Sylvian fissure.

The *præcentral sulcus* is directed upwards and distinctly backwards from the base of the ascending frontal convolution, which it limits anteriorly. Just before it reaches the level of the central point of the fissure of ROLANDO, it bifurcates into two horizontal limbs; the anterior and longer runs forwards, being slightly curved with the concavity downwards; the posterior, on the other hand, is directed upwards as well as backwards, and is extremely short, very rarely exceeding 2 mm. in length. The main stem of the sulcus presents a double curve, the upper half having a slight convexity backwards, while the lower half is markedly curved forwards. In this way the ascending frontal convolution is most narrow opposite the posterior superior limb of this sulcus, while below it widens broadly.

Superior Frontal Sulcus.—In December, 1883, Professor SCHÄFER published in the 'Journal of Physiology' an account of the brain of a Macaque Monkey, which was shown by Professor FERRIER at the International Medical Congress in London, in which account he drew attention to the existence of a small, but definitely marked, sulcus on the upper portion of the frontal lobe, having an antero-posterior direction, and dividing into two parts the surface of the brain between the upper end of the præcentral sulcus and the mesial margin of the hemisphere. This small sulcus lies just behind the line of direction upwards of the vertical stem of the præcentral sulcus, Professor SCHÄFER provisionally named this sulcus α .

We venture to think that this small sulcus is nothing less than the representative of the posterior extremity of the superior frontal sulcus of Man, and that, therefore, the portion of brain above it must be looked upon as the first or superior frontal con-

volution, and that that part which lies between it and the top of the præcentral sulcus is certainly the second or middle frontal convolution. Finally, all that portion of the cortex which lies below the anterior horizontal limb of the præcentral sulcus must at present be regarded as of uncertain denomination, since, for several reasons which need not be detailed here, the upper third of it would appear to belong to the middle frontal convolution, while there can be little doubt that the lower two-thirds are homologous with the third or inferior frontal convolution of Man. We will now return to the consideration of the evidence on which we ground the opinion that x is really the representative of the posterior extremity of the superior frontal sulcus in Man. This evidence we will consider under the following headings:—

1. Morphological significance as an important sulcus.
2. Functional significance as an important sulcus.
3. Anatomical form and direction.
4. Value as a determinant of localisation of function.

1. *Morphological Significance.*—We have already stated that Professor SCHAFER had previously observed the constancy of this small sulcus, which opinion is fully confirmed by our experience. He has further shown on a transverse section that it was marked by a distinct folding in of grey matter—the sure indication of an important sulcus.

2. *Functional Significance.*—We have found that if electrodes are applied to the sulcus itself no movement follows, whereas, if they be just shifted to either border, the characteristic effects about to be described are obtained. Here we have a functional proof that this sulcus, although small, definitely divides two portions of the motor cortex. These portions, we shall see directly, have a different function.

3. *Anatomical Form and Direction.*—With one exception, we have found that the most constant direction of this sulcus is antero-posterior, though frequently it is slightly oblique. The exception referred to was found in a pig-tailed Monkey (*Macacus nemestrinus*), in which specimen the sulcus was almost vertical, and consequently parallel, to the fissure of ROLANDO. Now we would submit that we have here faithful imitations of the varieties of form presented by the superior frontal sulcus in Man. For, as is well known, that sulcus is most frequently a more or less straight antero-posteriorly directed line, while sometimes, though more rarely, it resembles a miniature præcentral sulcus in possessing a vertical stem parallel to the fissure of ROLANDO, from the middle of which there runs forwards an antero-posterior limb. If, therefore, we regard this sulcus, x , as the posterior extremity of the superior frontal sulcus, its variations in form will be most easily understood. Finally, we would observe that the superior frontal sulcus in Man commences posteriorly, well behind the line of the præcentral sulcus, directed vertically upwards; in fact, it begins posteriorly in the middle of the ascending frontal convolution. It is just this first part of the superior frontal sulcus which, lying over the anterior half of the breadth of the ascending frontal convolution, forms the sulcus, x , of the Monkey's brain.

4. *Value as a Determinant of Localisation of Function.*—We do not intend here to dwell on this point, because its full importance will be seen in the subsequent description of our experimental results obtained by stimulation, but we will anticipate so far as to say that this sulcus enjoys the distinction of separating the seat of the primary representation of the upper limb from that of the lower, a distinction which, it need hardly be added, is of the first importance.

Thus we have been led to designate this sulcus, *x*, by the more definite term of superior frontal sulcus, and we trust that the above evidence will be accepted as tending to establish this position.

The Intra-parietal Sulcus.—There is one variation in the form of this sulcus which we will notice, not merely because, so far as we know, it has not been described before, but also because it alters slightly the localisation of certain movements of the digits. This variation simply consists in a bending horizontally forwards of the lower end of the sulcus towards the fissure of ROLANDO, from which, in extreme cases, it is sometimes separated by only 3 mm. of surface of grey matter. This is particularly marked in the pig-tailed Monkey (*Macacus nemestrinus*). We shall refer to this point further.

Ascending Parietal Convolution.—We would draw attention to the fact that the upper end of this convolution—in other words, that part which represents in Man the parietal lobule—is invariably subdivided vertically by a subordinate sulcus, which is situated at the junction of the anterior and middle thirds of the convolution and runs down parallel to the fissure of ROLANDO. The lower end of this subordinate sulcus extends most frequently to the level of *x*, the upper end always being separated from the margin of the hemisphere by a few millimetres of cortex.

With the foregoing observations we conclude the anatomical description of the part under consideration.

PREVIOUS RESEARCHES IN THE SAME DIRECTION.

We will now describe the results obtained by Professor FERRIER on stimulating the same region, quoting verbatim from his 'Functions of the Brain,' 1st edition, 1876.

From the account there given we have drawn up the following Table, showing the relation between his nomenclature of centres stimulated and ours, and in the third column the results he obtained.

We find, however, that the area of cortex in which the upper limb is represented extends a little further towards the fissure of SYLVIVS than he has indicated; it is difficult also to make out the exact position of our centres 2 and 2' in relation to his, but perhaps we may include them in his centre 6.

With these trifling differences, it will be seen that the broad facts in our account are practically identical with his, all minuteness of detail contained in the following pages being of course superadded by us.

TABLE 1.

| FERRIER'S Centre. | Authors' Cen.re. | FERRIER'S Resu't. |
|-------------------|--------------------|--|
| 3 | 12, 1' | Movements of the tail, generally associated with complex movements of thigh, leg, and foot, with adapted movements of the trunk by which the foot is drawn to the middle line of the body, as when the animal grasps with its foot or scratches its chest and abdomen. |
| 4 | 1 9 (?) 11 | Retraction, with adduction of opposite arm, palm of hand being directed backward. This action . . . is such as may be ascribed to the latissimus dorsi. |
| 6 | 3 3' 4 4' | Supination, flexion of forearm by which the hand is raised to the face. |
| a | 7 | Individual and combined movements of the fingers and wrist, ending in clenching the fist. Centres for the extensors and flexors of individual digits could not be differentiated, but the prehensile movements of the opposite hand are evidently centralised here. |
| b | 7' | |
| c | 8 | |
| d | behind 8' | |

We will now proceed to describe our method of experimentation, the results in full detail, and the generalisations which can be deduced therefrom.

Method of Experimentation.—The animal being thoroughly anaesthetised with ether, the left cortex was exposed *lege artis*, and the dura mater raised. A careful drawing was then made of the arrangement of the sulci, upon which was represented the position of the various points stimulated. The cortex, after being carefully dried to prevent diffusion of the current, was excited as follows. The apparatus employed was one DANIELL cell and an ordinary DU BOIS-REYMOND coil, but no attempt was made to equalise the make-and-break shock by means of a HELMHOLTZ wire. The electrodes were the ordinary platinum pattern, and were 2 mm. apart. The coil consisted of a primary bobbin wound round an iron core, with secondary bobbin sliding on a sledge over it, the distance between them being registered in centimetres, so that the strongest current would be at zero when the secondary coil completely covered the primary one. The primary current was interrupted by means of an ordinary NEEF's hammer, and the secondary currents with this arrangement were of a strength sufficient to produce the sensation of slight pricking on the human tongue when the secondary coil was at 8, i.e., 8 cm. from the primary. This very weak secondary current* was always employed so as to obviate the fallacy of diffusion. That this object was attained was obvious, for, if a certain movement was always obtained at one place, shifting the position of the electrodes for even one millimetre was sufficient to produce a totally different result.

* This was the weakest current which would produce a contraction in the muscles.

Since in all the brains we have experimented upon the positions of the principal sulci were perfectly constant, we regarded them as definite landmarks by which we could accurately ascertain in different brains the position of each centre.

Thus, commencing above in the ascending frontal convolution, we took as our first landmark the sulcus α , and in the horizontal line behind it we placed our two first centres, 1 and 1' (these to be explained directly, see fig. 1), and then placed opposite the level of the upper end of the præcentral sulcus the centres 3, 3'. Dividing the interval between these points (which in the average brain of the animals we employed, *i.e.*, nine out of the ten experiments, was 1 cm. in length) into two equal parts, we designated the point of division by the ciphers 2 and 2'. Below 3 and 3' we placed 4, 4' and 5, 5' at distances equal to that between 2, 2' and 3, 3'.

(The distance between the electrodes was 2 millimetres, and they were applied parallel to the longitudinal fissure at the points designated by the figures described above. These points or centres were about 4 mm. apart. Thus a considerable interval was left between the centres, which we explored in the same way, but we have not deemed it necessary to give the results of exciting the cortex at these intervals, since in every case they simply corroborated the effects obtained by stimulating the parts bounding them.)

We further found that the ascending frontal and middle-frontal gyri were so broad as to necessitate a vertical subdivision. To meet this contingency, we employ the plain figures for the centres of the posterior half, and figures dashed thus, 1', for the anterior half; both, of course, being on the same horizontal level.

On reference to Table 3 it will be seen that, with the exception of the fingers and thumb, the absolute number of times that any movement is produced is much less in the ascending parietal than the ascending frontal convolutions, and further that the representation of even the thumb and index decreases as the gyrus is explored from below upwards.

From our experiments it appears to us that the ascending parietal convolution has less claim than the ascending frontal to be considered as an area of extensive representation of movement. We have been so impressed with the importance of deciding this fact that we have usually explored the former gyrus with the current directly after the skull has been removed, and subsequently repeated our examination of it at various intervals during the experiment, so as to eliminate any error in the direction of loss of excitability of the cortex of this gyrus.

We would call attention to the extraordinary degree of symmetry which exists in all the Monkeys on which we have experimented, and also that this is not merely morphological, but also physiological. Although this is a matter of great interest, we cannot enter into it in further detail.

Before giving the detailed results of our work, we would lay down the following axioms founded on our experiments.

Axiom 1.—Viewing as a whole the "motor area" of the cerebral cortex for the

upper limb, as defined by Professor FERRIER, we find that the regions for the action of the larger joints are situated at the upper part of that area, close to the middle line, while those for the smaller and more differentiated movements lie peripherally at the lower part of the area.

Axiom 2.—As a general rule, *extension* of all the joints, particularly of the wrist and elbow, is the most characteristic movement of the upper part of FERRIER'S arm centre; while *flexion* is equally characteristic of the movements obtained by stimulating the lower part. Finally, between these two regions there is a small portion where alternate flexion and extension predominate, a condition to which we have given the name of *confusion*.*

We shall now proceed to give by means of tables the details of the experiments upon which the foregoing axioms are based.

In the following Table 2 we give general conclusions respecting the different movements of each joint obtained by stimulating the upper and lower half of the above-mentioned area.

TABLE 2.

| Upper part of Area. | Lower part of Area. |
|--|---|
| <i>Shoulder.</i> — Advancing. | Adduction (nil in lower $\frac{1}{4}$). |
| <i>Elbow.</i> — Extension (uppermost $\frac{1}{4}$), Confusion (second $\frac{1}{4}$). | Flexion (remaining $\frac{3}{4}$). |
| <i>Wrist.</i> —(1) Pronation (upper $\frac{1}{4}$). | Supination (lower $\frac{3}{4}$). |
| „ (2) Extension. | Extension (flexion at end of action). |
| Posterior part of Area. | Anterior part of Area. |
| <i>Digits.</i> — Flexion. | Extension. |
| <i>Thumb.</i> — Flexion (nil in upper $\frac{1}{4}$). | Flexion-Extension. |

Expansion of the foregoing Table (compare Table 2).

Shoulder.—In the above Table it will be noticed that rotation of the shoulder does not appear; this movement, though frequently observed, is but one of association. Rotation out occurs as the result of stimulating the lower three-fourths of the area, at least of that part which lies in front of the fissure of ROLANDO. In almost every case this rotation outwards was accompanied by flexion of the shoulder, so that the elbow was brought forward as well as rotated outwards.

Pure abduction was practically never seen by us, but it entered into the composition of the advancing movement, which we have shown to be characteristic of the upper fourth of the area. It will thus be seen that the movement of the shoulder, which we have called advancing of the arm, is neither pure flexion nor pure abduction, but a combination of these two.

Adduction is strongly characteristic of the movement of the shoulder in the lower

* Here both flexors and extensors are contracting at the same time, and consequently the joint is usually fixed in a median position, each group of muscles alternately dragging it in opposite directions.

half of the area. Indeed, a glance at Table 3 will show that it is almost the only movement in the lower third of the area, in which portion of the cortex, too, the joint is very feebly represented, movement of it only being met with once in ten times on stimulating the centre 5'. (See fig. 2 and Table 3.)

Adduction is the characteristic movement of the shoulder "centres" in the ascending parietal gyrus. Retraction (*i.e.*, extension with rotation out and some adduction) is especially represented in the anterior half of the upper third of the ascending parietal gyrus in the centres marked 9 and 11. (See Table 3.)

Circumduction was never seen.

Elbow.—We need only draw especial attention to the remarkably exact manner in which representation of extension of the elbow is limited to the upper fifth of the area experimented on, while flexion is equally the function of the lower three-fifths; and to the existence of a very important zone of cortex, where the phenomenon of confusion is represented, this forming a border-land between the regions of extension and flexion. The explanation of this zone of confusion is easy, since the representation of the elbow—the movements of which occur in only one plane—admits necessarily of closely limited localisation. (See Table 3 and fig. 3.)

Wrist.—In tabulating the movements of this joint, it was obviously necessary to separate its two distinct functions, *viz.*, pronation and supination, on the one hand, and flexion and extension on the other. We will first discuss the latter function, as by far the more important. On Table 3 it is seen how extremely constant is the movement of extension, and a moment's consideration will show that it is of fundamental importance, for it is clear that the delicate movements of the fingers could not possibly be performed with any degree of accuracy and force unless the wrist be previously fixed in moderate extension, and consideration of this fact also explains why the wrist is especially provided with powerful extensor muscles which act with considerable independence. (See fig. 4.)

In returning to the first-mentioned movements of the wrist, *viz.*, pronation and supination, we have only to add that, as might be expected, supination is most marked in the lower two-thirds of the area, since it is here that we have also flexion of the elbow, *i.e.*, bicipital action. The converse equally holds, *viz.*, that pronation is associated with extension of the elbow. This association is clearly the outcome of the two great classes of action in animal life, *viz.*, that of defence and that of feeding. The former of these is a coarse violent movement, and is naturally associated in the upper part of the region with the centres for the large trunk and leg muscles, while the latter, more delicate, is represented near to the centres for the face and mouth.* (See Table 2.)

Digits.—The representation of the fingers must be considered apart from that of the thumb, the movements of which are the most highly specialized in the limb.

* Similar ideas have been previously suggested by Dr. LAUNER BRUNTON, F.R.S. See *Brain*, vol. 4, p. 431.

We would first draw attention (see fig. 5) to the fact that the movement of simple extension is alone represented in the posterior extremity of the middle frontal convolution (according to our view), viz., at centres 12, 13, 12', and 13' (see Table 3 and fig. 5). It will be observed on Table 3 that in three instances we noted interosseal flexion in this region. Discussion of the relation between interosseal flexion and extension of the digits we shall enter into further on.

As regards the movement of flexion, we find that it is represented over the whole of the area which has been the subject of our investigations, with the exception of the centres above mentioned, viz., 12, 13, 12', and 13'. We have now to draw attention to some extremely important considerations concerning the relative representation of the movements of flexion and extension. We have just seen that pure extension was limited to certain centres; we have now to add that we have only seen pure flexion at centres 1, 1', 4, and 4'. We are not inclined to lay much stress on this limitation of the representation of the movements of pure flexion and extension, but we note the foregoing facts for the purpose of recording them.

We will now consider what appears to us the much more important co-operation of these two movements of flexion and extension of the digits. On this point we have obtained the exceedingly definite result that both movements are represented in the middle $\frac{1}{3}$ of the ascending frontal and parietal convolutions, and that, while in the ascending frontal convolution extension precedes flexion, in the ascending parietal convolution this order is reversed, and so extension follows flexion. In view of the fact that pure extension is only represented in 12, 12', 13, and 13', i.e., in those centres which are immediately in front of the middle $\frac{1}{3}$ of the ascending frontal convolution, we readily understand how it comes about that in the ascending frontal extension *precedes* flexion, whereas behind the fissure of Rolando it *follows* flexion. We have observed interosseal flexion to precede long flexion in three cases, and only in the centres 8', 2, 2', 12, and 3. It is obviously possible that this interosseal flexion of the digits, consisting of flexion of the metacarpo-phalangeal joints and extension of the phalangeal joints, should co-exist in perfect harmony with long extension of the digits. It must not be understood that in all the remaining cases interosseal extension of the phalanges leads the way for the action of the long extensors; indeed, in many instances it appeared to us that preliminary extension of the digits was so sharp and complete as to be explained only by rapid and perfect action of the long muscles. We must add, however, that, although this preliminary extension of the fingers was extremely well marked, it was of very brief duration, whereas the subsequent flexion was very powerful, and was maintained as long as the electrodes were applied to the cortex.

Although we have thus written at some length on the relation between flexion and extension of the digits, we do not consider that the results were sufficiently absolute to permit of our speaking dogmatically, and we hope that further research will ultimately solve this problem.

Thumb.—The limitation of the representation of the thumb in the cortex (see fig. 6) is a matter of great interest, considering that it is the most highly differentiated member of the body. The representation is limited to the ascending frontal and parietal convolutions. No movement could be elicited by stimulating the centres 1, 1', 5, and 11; to these we must add 2 and 2', as being centres in which the thumb was only exceptionally represented, *i.e.*, in one-third of the total number of cases for 2, and one-fifth for 2'. It is interesting to observe that the thumb obeys the same general rules respecting the relations of the movements of flexion and extension as do the digits; thus extension *precedes* flexion in the ascending frontal and *follows* flexion in the ascending parietal. The movement of opposition, which is, of course, the most highly differentiated one, was only obtained on stimulating the lowest part of the thumb area, *viz.*, the centres 7 and 5'; this is in perfect harmony with the general plan of representation, as we have found it to exist in the outer convex surface of the cortex. (See Axioms 1 and 2.)

We may here refer to the fact that in 5', in the ascending frontal gyrus, we obtained in two cases abduction of the thumb, whereas adduction was present in two cases in centre 7 in the ascending parietal; this is a further illustration of the fact that extension is represented in the ascending frontal, and flexion in the ascending parietal convolutions.

Before leaving the consideration of the representation of the thumb, we would briefly draw attention to the fact that the representation of the thumb extends lower down anteriorly, *i.e.*, just behind the præcentral sulcus, than that of any other part of the upper limb. Thus it is represented at centre 5', but not in centre 5, &c. (See Table 3.)

We employ throughout our paper this expression—Primary Movement—in a very definite sense, namely, to express that movement which is represented above all others at one particular spot in the cortex. This use of the expression is in harmony with Dr. HUGHLINGS JACKSON'S view, *viz.*, that cerebral localisation is in the main a matter of degree of representation of several movements, and not the close limitation of any one. We were brought to the necessity of closely examining this point, not from the considerations of theories, but by observing with what remarkable constancy the various joints of the limb took up movement in series according to the part of the cortex stimulated, and how invariably one joint would commence the action when we adopted a method of excitation which we may call instantaneous or minimal stimulation.

PRIORITY IN THE ORDER OF MOVEMENTS. (See fig. 7.)

We may now return with advantage to a detailed consideration of Axiom 1, *viz.*, that the larger muscles are represented in the upper part of the motor area for the upper limb, while the smaller ones have their centres in the lower part of that area.

It seemed to us highly important, as bearing on this point, to note the order of movement of the different segments of the limb,—in fact, the “march,” as it has been termed by Dr. HUGHLINGS JACKSON, of the nerve discharge,—since we consider that a complete series of observations of this kind would enable us to construct a definite scheme which would show at a glance where certain primary movements are really centralised.

By this we mean that we applied the electrodes to the cortex just long enough to evoke movement in one joint only, and then noted which moved first, and in what direction. This first movement we considered to be the primary or fundamental movement in the given portion of cortex stimulated. (The current employed in every case was only just adequate to produce such movement, and the secondary coil was usually 10 cm. distant from the primary coil.)

On fig. 8 is exhibited that joint in which primary movement occurs in each portion of the area.

In taking each joint separately, it is found that the shoulder presents priority of movement in the centres 1, 1', 11; while the wrist is the first to move when we stimulate the centres 2, 2', 12, 13, 3'. It is important to notice that the elbow does not present any absolute priority of movement over the other joints, for its only approach to priority is seen at centres 8, 8', and 9, where it is associated with, and shares this action synchronously with, other joints, viz., the thumb and wrist. With regard to the fingers alone, the same thing is to be observed, viz., that they are not represented in primary movement in the cortex, and indeed are only associated once with a true primary movement, viz., in centre 12. Starting now with the series of joints moved as represented schematically in fig. 8, we will take up the order in which the other joints of the limb are secondarily moved.

Tabulating these joints in the order of centres stimulated, we have the following list:—

Ascend. Frontal
Convolution.

Centre.

Order of Joints moved.

- | | | | |
|-----|---|--------------------------------|----------------------|
| 1. | Shoulder | { elbow, wrist, fingers. | . |
| 1'. | Shoulder, elbow | { wrist, fingers. | |
| 2. | Wrist, fingers, elbow, shoulder. | | |
| 2'. | Wrist | { fingers } { elbow } | shoulder. |
| 3. | Thumb, fingers, wrist, elbow, shoulder. | | |
| 3'. | Wrist | { thumb } { fingers } | elbow, shoulder. |
| 4. | Thumb, wrist, fingers, elbow, shoulder (4). | | |
| 4'. | Thumb | { fingers } { wrist } | elbow, shoulder (2). |
| 5'. | Thumb, fingers, elbow | { wrist (1), shoulder (1). | |

Ascend. Parietal
Convolution.

- | | | | |
|-----|-------------------------------------|-----------------------------|----------|
| 11. | Shoulder | { wrist } { elbow } | fingers. |
| 9. | Thumb } Elbow } | fingers, wrist, shoulder. | |
| 8'. | { thumb } { wrist } { elbow } | { shoulder } { fingers } | |
| 8. | { thumb } { elbow } | fingers, wrist. | |
| 7'. | Thumb, elbow, shoulder (1). | | |
| 7. | Thumb, fingers, wrist, elbow (1). | | |

Middle Frontal
Convolution.

- | | |
|-----|---|
| 12. | { Wrist } elbow } { Fingers } shoulder } |
| 13. | Wrist, fingers, elbow, shoulder. |

The figures in parentheses show the greatest number of times out of a total of 10 in which a movement in the joint was produced.

As is shown in this Table, the sequence in the movement of the joints is fundamentally similar to that which had been arrived at from clinical observation by Dr. HUGHLINGS JACKSON in cases of epilepsy, in which he had recorded the "march" of the movements of the joints.

As all the facts which we have accumulated on the subject of the primary representation of movement of joints and the representation of secondary, &c., movements are collated in a very demonstrative manner in the accompanying figs. 7 and 8, a detailed description is hardly called for, but we cannot leave this pathologically very important subject without dwelling for a moment on some of the more salient features of our results.

We have already pointed out on page 163 why the shoulder, wrist, and thumb are the joints, *par excellence*, in which primary movement takes place.

We will now, therefore, discuss the march, *i.e.*, the order in which the movements of the various joints follow each other after the primary movement. The first and most fundamental fact concerning the successive invasion of the various joints has already been determined by Dr. HUGHLINGS JACKSON, *viz.*, that when a movement emanating from the cortex, *e.g.*, of the upper limb, begins in the shoulder it proceeds downwards, involving successively the elbow, wrist, and fingers; and inversely, when it begins in the thumb and fingers, the "march" proceeds up the limb. We are here referring to movements presenting the characters of deliberate purpose, "voluntary" efforts, which also can be evoked by electric stimulation of the cortex, besides being exhibited in convulsive and epileptiform seizures. The observation of these movements as produced in our experiments has enabled us to form certain definite generalisations concerning the order of their march. Among these generalisations, the following appear to us to be the most important.

1. Movement of the upper limb, commencing with the shoulder, is not completed by a movement of the thumb; and, while this result is obtained at the extreme upper limit of the area, on the other hand, we have the exact converse at the lowest limit, *viz.*, movement of the limb, commencing in the thumb, and ultimately involving the elbow, which is not completed by movement of the shoulder. In fig. 8 is shown diagrammatically the order of the march of movements occurring at each point in the area for the upper limb.

2. We wish next to point out the very remarkable constancy in the order of march in the centres 2, 2', 13 (fig. 8). Here we are dealing with the very nucleus of the upper limb area, *i.e.*, that part of it in which the most frequent and most ordinary movement of the limb is represented, *viz.*, preliminary fixation of wrist in extension, intended, as we think, for the purpose of permitting accurate movement of the digits; following this, flexion of the digits; next flexion of the elbow and subsequent adduction and rotation out of the shoulder, producing the complex movement which has been popularly styled the hand-to-mouth action, and which is unquestionably one of the most

important of the limb. The remarkable way in which this movement is represented in a nearly horizontal line on the cortex must not be overlooked.

Finally, fig. 8 illustrates most clearly how the mode of march is in harmony with the representation of primary movements in the various points in the area. Thus the movements consequential to the first movement obey the law we have already dwelt upon, viz., that the joints are represented from above down in the area, in the order of shoulder, elbow, wrist, digits, and thumb. The truth of this statement is rendered very evident when fig. 7 is compared with fig. 8.

To summarise briefly the facts contained in the foregoing pages, we consider:—

1. That sulcus *x* (SCHAFER) corresponds to the superior frontal sulcus of Man.
2. That the muscles of the upper limb are progressively represented from above downwards, in the outer or convex surface of the hemisphere, in the order of their size and the movements of the joints: in the order of shoulder, elbow, wrist, finger, thumb.
3. That the joints are moved in the order of shoulder, elbow, wrist, and hand when the highest part of the area is stimulated, and in the converse order—thumb, fingers, wrist, elbow, shoulder—when the lowest part is excited, whilst between these extreme points the sequence of movement is commenced by a middle joint, i.e., the elbow (incompletely), in the ascending parietal convolution, and the wrist to a very large extent in the ascending frontal.
4. That with regard to the quality of movements of the different joints represented in the cortex, the *shoulder* presents the following sequence from above down: advancing, abduction, rotation out, adduction; the *elbow*: extension, confusion, flexion; the *wrist*: extension, flexion, and pronation, confusion, supination. In the fingers and thumb the sequence is altered, and we have, broadly, extension anteriorly and flexion posteriorly. (For details refer to diagrams.)
5. That there is no *absolute* line of demarcation between the area of localisation in the cortex of one movement and that of another; each movement having a centre of maximum representation, this gradually shading off into the surrounding cortex.

TABLE 3.—*Résumé.* (Ten Experiments; one incomplete.)

| <i>Thumb.</i> | <i>Fingers.</i> | <i>Wrist.</i> | <i>Elbow.</i> | <i>Shoulder.</i> |
|--|---|-----------------------------|------------------------------------|--|
| 1. Nil | <u>Flexion</u> , 5. | <u>Extension</u> , 3. | <u>Extension-flex.</u> , 90°, 9. | <u>Advancing abd.</u> , 10. |
| 1'. Nil | <u>Flexion</u> , 7. | <u>Extension</u> , 3. | <u>Extension</u> , 10. | <u>Advancing abd.</u> , 9. |
| 2. ? <u>Flexion-extension</u> , 4. | <u>Ext.-flexion</u> , 10. | <u>Extension</u> , 3. | <u>Flexion-confusion</u> , 5 to 5. | <u>Rot. out. add. abd.</u> , 7. |
| 2'. <u>Slight extension-flexion</u> , 3. | <u>Ext.-flexion</u> , 9. | <u>Extension</u> , 7. | <u>Flexion-confusion</u> , 5 to 5. | <u>Rot. out. add. abd.</u> , 8 |
| 3. <u>Slight ext.-flex.</u> , 5 to 2.* | <u>Ext.-flexion</u> , 1 to 8. | <u>Extension</u> , 4. | <u>Flexion</u> , 10. | <u>Rot. out. add. abd.</u> , 8. |
| 3'. <u>Ext.-flex.</u> , 2 to 1. | <u>Ext.-flexion</u> , 4 to 2. | <u>Extension</u> , 5. | <u>Flexion</u> , 5. | <u>Rot. out. add. abd.</u> , 6. |
| 4. <u>Ext.-flex.</u> , 2 to 4. | <u>Flexion</u> , 5. | <u>Ext.-flex.</u> , 3. | <u>Flexion</u> , 9 | <u>Add. rot. out.</u> , 5. |
| 4'. <u>Ext.-flex.</u> , 3 to 5. | <u>Flexion-ext.</u> , 8. | <u>Extension</u> , 2. | <u>Flexion</u> , 9. | <u>Add.</u> 4. |
| 5. Probably <i>nil</i> . | 1 exception, same as 4'. | <u>Extension</u> , 1. | <u>Flexion</u> , 2. | <u>Add.</u> , 1. |
| 5'. <u>Ext-opp-abd.</u> , 8. | <u>Ext.-flexion</u> , 4. | <u>Extension</u> , 1. | <u>Flexion</u> , 2. | <u>Add.</u> , 1. |
| 7. <u>Flexion-add-opp.</u> , 6. | <u>Flexion-ext.</u> , 4. | <u>Slight flexion</u> , 1. | <u>Slight sup.</u> , 1. | Nil. |
| 7'. <u>Slight flex-ext.</u> , 2. | <u>Inters. flexion</u> , 1. | <u>Extension</u> , 1. | <u>Flexion</u> , 1. | <u>Rot. out.</u> , 1. |
| 8. <u>Flexion-ext.</u> , 8 to 1. | <u>Flexion-ext.</u> , 7. | <u>Sup-pron.</u> , 2. | <u>Flexion</u> , 4. | <u>Rot. out.</u> , 1. |
| 8'. <u>Flexion-ext.</u> , 8. | <u>Flexion-ext.</u> , 6. | <u>Supination</u> , 1. | <u>Flexion</u> , 3. | <u>Abd. confusion</u> , 1; <u>add. retr.</u> , 1. |
| 9. <u>Extension</u> , 1. | <u>Ext.-flexion</u> , 3. | <u>Extension</u> , 2. | <u>Flex-conf.</u> , 2. | <u>Add.</u> , 2. |
| 11. Nil | <u>Flexion-ext.</u> , 2. | <u>Extension-flex.</u> , 2. | <u>Conf.-flexion</u> , 3. | <u>Adduction with retraction</u> (<u>Lat. dors.</u>), 4. |
| 12. Nil | <u>Extension</u> , 6; <u>inters. flex.</u> , 1. | <u>Extension</u> , 6. | <u>Pronation</u> , 1. | <u>Rot. out. abd.</u> , 5. |
| 12'. Nil | <u>Extension</u> , 1; <u>inters. flex.</u> , 2. | <u>Extension</u> , 1. | <u>Confusion</u> , 2. | <u>Rot. out.</u> , 1. |
| 13. Nil | <u>Extension</u> , 2. | <u>Extension</u> , 3. | <u>Supination</u> , 1. | <u>Rot. out. add.</u> , 2. |
| 13'. Nil | <u>Extension</u> , 1. | <u>Extension</u> , 1. | <u>Supination</u> , 1. | <u>Rot. out. add.</u> , 2. |

N.B.—The sequence of movement is indicated by the amount of underlining, thus:—The primary movement is denoted by one line, the secondary by two lines, and the third and fourth by three and four lines respectively.

Note.—The numbers following the record of each movement indicate the number of times that any action was obtained by stimulating the given centre. As before mentioned, we do not here give the results of stimulating the centres indicated by fractions, i.e. the areas of cortex intervening between the above enumerated centres, because they simply corroborated the observation that the representation in one centre gradually merged into that of the next.

* Adduction in one case.

VII. *Supplemental Note on Polacanthus Foxii, describing the Dorsal Shield and some Parts of the Endoskeleton, imperfectly known in 1881.*

By J. W. HULKE, F.R.S.

Received December 14, 1886,—Read January 18, 1887

[PLATES 8, 9.]

IN a former paper,* descriptive of the type remains of *Polacanthus Foxii*, some account was given of its dermal armour, but the description was unavoidably very incomplete, owing to the extremely fragmentary state of the parts originally composing it. Broken up by its discoverer into pieces small enough for convenient stowage and transport in bags from the cliffs to the village of Brighstone, two-and-a-half miles distant, and then uncared for during fifteen years, the great dorsal shield, when after Mr. Fox's death his collection was acquired by the British Museum, was represented by several hundreds of disconnected pieces, many of these being of less size than one cubic inch. It was also evident that many had been lost. In this mutilated condition the reconstruction of the shield appeared hopeless, but at length, under the guidance of the heads of the Palæontological Department, this has been accomplished by Mr. HALL and Mr. BARLOW ("Masons"), who brought to the task a painstaking perseverance and skill worthy of the highest praise. Although now, doubtless, much less complete than when laid bare in the cliff by Mr. Fox, the reconstruction (which has consisted strictly in a faithful reunion of the disconnected scattered fragments) renders very intelligible the discoverer's first impression, viz., that "he had before him the carapace of a gigantic turtle," and it confirms his opinion of the position of the shield, viz., that it covered the rump and loins. The dimensions of the shield, given by Mr. Fox in a MS. note, 3 feet 3 inches by 3 feet, were taken roughly in the cliff before the shield was broken up. In its restored condition its breadth is 108 centims. in front, 105 centims. at its middle, and 48 centims. posteriorly, and its length is 90 centims.

The relation of the shield to the pelvic bones makes it evident that the carcass was lying on its belly when it sank into the ooze, and that the shield was later crushed down upon the endoskeleton and flattened out.

In its present state the outline of the shield forms a long oval figure, from which an anterior segment has been removed through a line parallel to its shorter axis. It

* 'Phil Trans.,' 1881 (vol. 172), p. 653.

is now evident that the pieces which I described in my paper of 1881 were not all integers of the value of separate scutes, entering by overlap, or other arthrodial articulation, into the composition of the shield, but (with the exception of the γ spines) were pieces originally synostosed in a continuous sheet, in which no traces of suture or other marks of primitive distinctness are discernible.

Near its lateral and posterior border the shield is thicker than at the middle, where it overlies the vertebral column, a fact noticed by Mr. Fox. The form of these borders is that of a smoothly-rounded lip separated from the upper surface by a narrow sunken groove. The continuous sweep of the posterior border is interrupted by a wide deep notch, having at its centre a projecting part that overhung the root of the tail. At each side of this projection is a narrow cleft; whether natural, or a crack produced by the yielding of the shield under compression, is uncertain. The anterior differs from the other borders of the shield by its attenuation to a thin edge. This circumstance, together with the truncated form of the border, suggests that the part we actually possess does not represent the complete dorsal mail, but only a posterior segment, between which and an anterior part, now lost, covering the thorax, a flexible junction may have existed somewhat like that present in the plastron of certain Chelonians.

The upper, or exterior, surface of the shield is richly ornamented. It exhibits in a highly satisfactory manner the position and distribution of the pieces described by me in 1881 as "*tuberculated*" (α) and "*keeled*" (β) scutes. The former (α) compose the general groundwork co-extensive with the whole area of the shield, which is closely studded with hemispherical tubercles averaging 5 to 1.5 centims. across their base. The β pieces, characterised by a keel-shaped elevation rising out of a circular or elliptic depression, are grouped in four longitudinal rows occupying each lateral half of the shield. The highest and stoutest part of the keel is always posterior. The largest keels form a sub-marginal lateral row; and the smallest a paired sub-median series, one in each pair lying on each side of the middle line of the shield over the vertebral column. The central elevation in this latter series resembles a low blunt cone, with circular or oval base, rather than a keel. The keels of intermediate size compose two less regular rows placed between the sub-marginal, lateral, and the sub-median series. It is now certain that the large spines (γ) do not constitute any part of the shield we possess; and since, for reasons given in 1881, they appear excluded from the caudal mail, they would seem to have occupied an anterior region of the trunk, a supposition which derives some additional probability from the position of the series of similar spines in the type-specimen of *Hylæosaurus* preserved in the British Museum.

Thin sections of the shield, mounted in Canada balsam, show an osseous structure. Near the inner surface the arrangement of the trabeculæ conforms to that of the decussating bundles of fibrous tissue observable in the cutis vera of existing Lizards. Near the outer surface sections, vertical to and parallel to this surface, show an

areolated or cancellated arrangement of the bony tissue; and here may be seen numerous vascular canals surrounded by concentrically placed lamellæ.

Thus the shield doubtless in part represents the cutis vera. It must have been invested by a vascular membrane analogous to the periosteum of the endoskeletal bones, and externally this was overlaid by an epidermal covering which sheathed the keels and spines.

Upon the inner surface of the shield are apparent in the middle line the sacrum and a series of lumbar vertebræ synostosed and forming a rigid bony rod (as described in 1881). The lower transverse processes, imperfectly known to me in 1881, are now well shown.

Those of the 2-5 sacral vertebræ average 14-15 centims. in length. Their stout, expanded, distal ends abut against the median surface of the ilia. The lower transverse processes of that which I have called the first sacral vertebra are less stout, and their direction differs slightly from that of the others; their connection with the ilia is also less evident. It is, therefore, possible that this vertebra may better claim to be the last of the lumbar series. (In *Iguanodon* this vertebra is often found synostosed with the first sacral, and so functionally composing part of the sacrum.)

Five ribs on the right side still retain nearly their normal relations with the lumbar vertebræ. They progressively shorten from before backwards, their lengths decreasing from 28 to 25 centims.; these are approximate measurements, because the distal ends of the ribs merge into and are lost in the inner surface of the shield without distinct indication of their actual termination. In their vertebral halves these ribs exhibit well the inferior ridge which gives a triquetrous figure to their cross-sections, and must have greatly increased their strength. The vertebral ends of the ribs are crossed superiorly by longitudinally disposed bundles of slender bony rods. These are manifestly ossified tendinous and ligamentous structures, similar to those which I have mentioned as occurring in *Hypsilophodon Foxii*, and to those in *Iguanodon* lately described by Mr. DOLLO.

The pelvis, of which in 1881 little was recognisable, is now worked out. The acetabula are well displayed; their large size immediately attracts the eye. The ilia are so blended with the shield that their exact form is not discernible. So far as slight textural differences of the surface warrant an inference, I am disposed to think that the præ-acetabular was longer than the post-acetabular portion, but I speak with reservation on this point. As in many Dinosaurian ilia, the pubic part of the acetabular arc of the ilium forms a strongly marked angle, from which abruptly rises the lower border of the præ-acetabular process. The remains of the os pubis are too fragmentary to give the shape of this bone, but I think that indications are recognisable of its division into a *præ-pubic* and a *post-pubic* part. Other specimens must, however, decide this.

The ischium is better preserved, the left being nearly entire. It has a compressed doubly-curved figure of simple form, decreasing from a breadth of 15 centims., where it

joins the ilium, to 4·7 centims. at its ventral or mesial end. Its direction appears to be nearly transverse to the long axis of the trunk, and not almost parallel to this, as in *Iguanodonts*. Whether the ischia actually met in symphysial union cannot be ascertained from this specimen, as the mesial end is missing.

EXPLANATION OF PLATES.

PLATE 8.

Dorsal View of Shield.

| | | |
|-------------------------------|--|-----------------------------|
| <i>L. v.</i> Lumbar vertebræ. | | <i>Isch.</i> Right ischium. |
| <i>C. v.</i> Caudal vertebræ. | | <i>Isch.'</i> Left ischium. |

PLATE 9.

Ventral View of Shield and Pelvis.

| | | |
|---|--|-----------------------------|
| <i>L. v.</i> Lumbar vertebræ. | | <i>Isch.</i> Right ischium. |
| <i>C. v.</i> 1st caudal vertebra. | | <i>Isch.'</i> Left ischium. |
| <i>S.</i> 1, 2, 3, 4, 5. Sacral vertebræ. | | <i>r.</i> Ribs. |
| <i>a.</i> Acetabulum. | | <i>t.</i> Ossified tendons. |

VIII. *On the Structure and Life-History of Entyloma Ranunculi* (BOXORDEN).

By H. MARSHALL WARD, M.A., F.L.S., Fellow of Christ's College, Cambridge, and Professor of Botany in the Indian Civil Engineering College (Forestry School), Cooper's Hill.

Communicated by W. T. THEISELTON DYER, C.M.G., F.R.S., Director of the Royal Gardens, Kew.

Received October 12,—Read November 25, 1886.

[PLATES 10-13.]

THE attention of mycologists has been long directed to the study of the Ustilaginæ, not only on account of their morphological peculiarities, but even more especially because the economic questions arising from their relations to our crops, &c., have assumed such proportions as to force this group of parasites far into the foreground. Interesting and important as are the parasitic habits of the Ustilaginæ, however, and much as they have been investigated, it has to be admitted that we know as yet very little about them. Two or three of the most common forms, it is true, have been so often studied by different observers that they may be regarded as worked out sufficiently to allow of our regarding them as types; but it needs no very extensive acquaintance with the group to satisfy ourselves that the best known forms are not the simplest, and that much still remains to be accomplished in this large group. It is not only that the Ustilaginæ are so minute, but they are so peculiarly modified, and so specialised as parasites, that the most careful observation is necessary in making out the numerous points in their structure; in addition, observers still differ considerably as to the interpretation of some of the facts of structure which are established.

Taking the most recent systems* of classification, we may regard the Ustilaginæ as comprising the following genera, *Ustilago*, *Tilletia*, *Sorosporium*, *Urocystis*, *Schizoneura*, and *Entyloma*, and so far shall be in accordance with all the modern authorities; when we come to such genera as *Geminella*, *Sphacelotheca*, *Doassansia* (CORNU), and *Graphiola* (FISCHER) and some others, the matter becomes more complicated, and special investigations are still needed to determine the limits of the genera and group. *Entyloma*, however, is a well-established genus,† and now includes some fifteen or

* E.g., DE BARY, 'Morphol. d. Pilze,' p. 186, and WINTER, 'RABENHORST'S Kryptogamen-Flora,' p. 80.

† DE BARY, 'Botan. Zeitung,' 1874, p. 101. SCHROEFER, in CORN'S 'Beiträge Biol. Pflanz.,' vol. 2, pp. 368 and 489.

sixteen species. They are parasitic in the mesophyll of the leaves of various plants, and are characterised by producing rounded resting-spores as intercalary swellings on the very fine, septate, intercellular mycelium; these spores germinate like those of *Tilletia*. In *Tilletia*, however, the resting-spores form dense powdery aggregates, which is not the case with the more isolated spores of *Entyloma*.

Of the various species of this genus referred to above, three are described as occurring in the leaves of species of *Ranunculus*. One of these—*Entyloma ranunculi*—appears to be extremely common in some places, and has a wide distribution; its resting-spores have often been described, and it is regarded by all the authorities as a well-marked species. It has, however, so far as I know, not been specially investigated in detail, and, in addition to the other facts contained in the following memoir, it is of interest to have observed the germination of the conidia for the first time; the infection experiments are also new, and consequently important, and they establish beyond doubt the relations of the conidia to the *Entyloma*.*

I now proceed to the description of my own observations, the completion of which has been rendered possible by an outbreak of the disease which the fungus induces on a large patch of densely crowded plants of *Ranunculus Ficaria* during the spring and early summer of this year; this patch of diseased plants was so favourably situated, and the fungus spread so rapidly and in such enormous numbers on it, that I was enabled to observe and record some facts of considerable interest respecting the origin and spread of the epidemic due to the action of the parasite. Moreover, material was to hand in abundance and in excellent condition, and the circumstances were so fortunate that it was possible to note day by day the symptoms of the disease, and the progress, climax, and decline of the epidemic. I mention this expressly because it is not sufficiently recognised how necessary is the study of the diseases of plants in the field—clinically, as it were—as well as in the laboratory.

The patch of *Ranunculus* referred to above extended some distance along the course of a conveniently situated damp ditch: during February and March thousands of young fresh green leaves sprang up, and in April the ground was densely carpeted with them; the leaves were so closely packed that the ditch appeared full of them. The ditch itself runs nearly due north to south, and is only damp as a rule; it becomes filled with water during heavy or continuous rain, however, and the water

* With respect to these conidia, it appears that they have been described at various times as the spores of other species of Fungi, and that WINTER first suggested their connection with *Entyloma*. Then SCHROETER observed conidia in connection with *Entyloma serotinum*. (SCHROETER, *loc. cit.*, pp. 369 and 438.) WINTER ('Kryptogamen-Flora,' p. 113) says:—"Sporidienbildung auf der lebenden Nahrpflanze," which conveys the impression that the resting-spores germinate in the leaf and bear "sporidia" on their promycelia. The bodies here referred to are true conidia, however, as is clear from my observations, and are developed independently of the resting-spores. Moreover, this is the first time the germination of the conidia of an *Entyloma* has been followed and described (*cf.* DE BARY, 'Morphol. d. Pilze,' p. 194), thus placing their connection with the *Entyloma* beyond doubt, and explaining their nature as true conidia.—May 5, 1887.

runs off rather slowly to the south. A fairly dense growth of hazel and other trees overshadows all, and in the summer but little sunlight reaches the plants in the ditch after noon: the morning sun nevertheless reaches the plants under and through the trees during the earlier months named. One consequence of all this is that the leaves of the *Ranunculus* were very succulent, bright, and long-stalked, and, as already mentioned, appeared to fill up the hollow of the ditch.

Towards the middle of April the bright green glossy leaves of the *Ranunculus* plants in a certain part of the above patch were noticed to be slightly spotted with white flecks, which increased in size and number day after day until before the middle of May a long tract—several yards—of the thickly growing leaves were infected and thus spotted.

Before describing the phenomena more closely, and simply speaking of the white flecks as the chief obvious symptom of the diseased condition, I may call attention to one or two points which seem significant. *Ranunculus Ficaria* is an extremely common plant all over the neighbourhood of Englefield Green, and nevertheless I have failed to find the white spots on leaves in many places near. Nor is this all; hundreds of plants on the eastern side of the garden (the ditch runs along the western side) have been examined, and no traces of the spots found, and even in the ditch referred to none of the plants in the northern two-thirds of its length were spotted as described. The disease—the epidemic, I will say—commenced on a few plants in April, and spread southwards for several yards during April and May. I account for this as follows:—The easily spread spores (conidia) of the fungus causing the disease were transferred by wind, and especially by water flowing southwards in the ditch during the rains occurring at various periods in April: the wind, as I had occasion to notice, was chiefly from the north and east at these times, hence the immunity of the plants in the northern parts of the ditch and on the other side of the garden. Even the fact of a south-west or west wind occasionally does not contradict the conclusion when all the circumstances are known, for a high bank and hedge lie to the westward of the ditch, and the hazel trees mentioned above would screen other parts of the garden.

An extraordinarily severe outbreak of the white spots occurred over the patch during the period May 6th to 12th, and it was then I noticed particularly how the epidemic spread to the south, and not to the north; the period referred to was remarkable for very warm “steamy” mornings and very bright noons. A storm broke over Englefield Green on Saturday, April 24th, and the ditch was flooded and overflowing for several hours, all the *Ranunculus* plants being bent downwards towards the south when the water had passed over and through them: that the flooding in question distributed the spores which caused the sudden and extensive outbreak on May 6th to 12th will hardly be questioned after what follows, for I shall show that it requires from a fortnight to three weeks to develope a white disease-spot in the leaf from a spore germinated on its epidermis.

I now pass on to a more detailed description of the white "disease-spots" on the leaves (Plate 10, fig. 1). On its first appearance the spot is pale and greenish, and not sharply marked off from the surrounding tissue, and it requires close watching to be sure when it first becomes visible to the unaided eye; shortly afterwards, the central parts of the enlarging fleck are pure white, resembling powdered chalk, especially when the air is warm and still, and the conidia to be referred to have accumulated in large quantities. As the white speck ages and enlarges centrifugally, it turns more ash-coloured or yellowish in the centre, and finally becomes brownish, or even dark brown, and the patch of tissue is dead.

These spots appear on both sides of the leaves, and are alike, except that the white stands out more sharply in contrast with the darker glossy green of the upper side of the leaf. On a warm, still morning, it is possible to collect relatively large quantities of the white chalky powder (conidia) from the more active flecks, and it will be seen how important this must be in the reproduction and spread of the fungus causing the infection.

The white flecks are confined to the mesophyll of the leaf, and can be seen sharply bounded by the vascular bundles of the venation—for instance, in the fork whence two chief veins diverge—whereas they fade imperceptibly into the green of the mesophyll. The leaf is not thickened at the infected spots, but it is very noticeable, as the spot increases in age, that it becomes thinner and dries up or rots; in the former case cracking and tearing away from the healthy tissue, and, in the latter, falling down as putrid shreds (fig. 1). The difference depends on weather. In both cases the bits may soon disappear, and the leaf look as if a piece had been nibbled out.

It will thus be seen that the diseased condition is confined to a given area; the spots are local centres, and do not spread indefinitely over the leaf. I have counted 57 on a leaf less than $1\frac{1}{2}$ inches broad and long, and many more can co-exist on that area. In some cases spots run together as they age. As I shall show later, each spot spreads from one centre only, i.e., from a stoma through which a germinal tube from a spore has passed; the stomata are on both sides of the leaf.

A curious, though by no means isolated, phenomenon is presented in the case of old leaves, which have shown the spots at a late stage of their life, and then turned yellow before the spots reach their matured condition: this is the existence of a vivid green ring around the spot, and is, without doubt, due to the mycelium of the fungus keeping the cells active after their neighbours are dead. I have noticed the same fact in the case of other parasitic fungi.

Sections through a young white spot show that a very delicate, copiously branched, mycelium exists between the cells of the mesophyll, both in the palissade and spongy tissue (figs. 7–14). Closer examination shows that the mycelium is segmented at rather long intervals, but the septa are very difficult to observe without reagents, owing to their thinness and that of the outer walls, and to the dense, finely granular

protoplasm in the hyphæ. If the sections are made through somewhat more advanced spots, the following additional peculiarities are noticeable (fig. 11). The mycelium has increased, and now sends branches into the lacunæ beneath the stomata. These branches fill up the interspace, and at length project through the orifices of the stomata in dense tufts or pencils (figs. 2, 6, and 11). The hyphæ are also seen to be mingled with numerous small spherical bodies—the resting-spores of the fungus. Subsequently the number of these spores increases enormously, until, in old spots, every nook and cranny between the cells is packed with them. Meanwhile, the pencils of hyphæ projecting to the exterior have produced innumerable colourless conidia from their free ends (figs. 3, 4, 5, 12, 13). It is these tufts and conidia which give the white powdery appearance to the spots.

Having thus given a general account of the fungus, I may proceed to describe further details as to the intercellular mycelium. It is not difficult to observe that at the margins of the spots (in the mesophyll tissue) the tips of the hyphæ are extending radially in all directions, branching as they do so, and forming septa behind the apices. Where the hyphæ pass along the wall of a cell, they frequently form flattened short branchlets or tufts, closely appressed to the outside of the wall of the cell (fig. 7), i.e., on the side bounding the lacunæ. These flattened tufts of branchlets are strikingly suggestive of haustoria, though they do not obviously pierce the wall. The hyphæ appear never to be intracellular. In some cases, with the aid of reagents, I have convinced myself that the attachment of these haustorium-like branchlets to the cell-wall is very close, and cannot help suspecting that either fine threads of protoplasm pass out to them from the sac of protoplasm inside, or that they send such fine threads through the cellulose; it has so far been impossible to place this beyond doubt, however. Although the hyphæ do not penetrate into the cavity of the cell, they can pass in the primary cell-wall (the middle lamella), and so force their way between two contiguous cells. Good sections show this distinctly, though, owing to the delicacy of the hyphæ, they are not easy to obtain. Moreover, as will appear clearer shortly, the tips of the hyphæ can make their way to the exterior between contiguous cells of the epidermis (figs. 11 and 14).

Following those hyphæ the tips of which protrude through the stomata, their ends are found to give rise to delicate colourless conidia by abstriction. Taking a given hypha, it grows out into the damp air or water, and its tip swells up slightly into an ovoid body which may lengthen considerably or not before it is separated off as a very delicate colourless conidium, with an extremely thin cell-wall and finely granular and vacuolated protoplasmic contents, in which minute brilliant oil-like drops are suspended.

In some cases, apparently in drier warm weather, the protruding hyphæ are relatively short, and the conidia ovoid or slightly reniform (fig. 3); in other cases, apparently in wet weather, and certainly in water (fig. 2), the hyphæ may protrude twice as far before the conidia are abstricted, and the latter are then longer, more

curved, and relatively thinner (fig. 5). Under such circumstances a conidium may be seen to germinate before it falls off from the hypha; or the hyphæ may go on growing longer and longer for many hours, to end at last, however, by forming long conidia at the extremity, the intermediate part dying off (fig. 2, *a*). Such abnormally long conidial segments are easily obtained by allowing the tufts to grow out from the stomata of cut-off pieces of epidermis suspended in water; the tufts thus produced are curiously suggestive of the so-called *Rumularia*, *Cercospora*, &c., of authors. The tufts of conidia are like *Glæosporium Ficariae* (BERK).

The normal conidia are club-shaped or long ovoid bodies, slightly curved, and more pointed at the attached end. They were to be obtained in any quantity, on the leaves in the ditch referred to above, in May, and I was able to obtain pure sowings of them with ease, by removing them lightly with a fine camel-hair pencil, and thus not only to observe all stages of germination, but also to infect clean plants with certainty.

Sown in water, in hanging drops kept over damp cells, the conidia germinate readily under favourable conditions. I have noticed that in many cases a sowing of two or three conidia in a drop remained unaltered for several days, the conidia finally dying off, or one or two germinating at last; whereas, in drops containing some dozens of the conidia, the germination sometimes followed more rapidly and certainly. At first I put the phenomenon down as probably due to temperature; further experience leads me to doubt the accuracy of that conclusion. Another point I am convinced of: conidia sown in a drop of water on a leaf of the living plant germinate more readily than those in a similar drop on glass. Nevertheless, it has been sufficiently easy to get the conidia to germinate in rain water, and I have seen hundreds, and perhaps thousands, of them in all stages of germination.

To describe a concrete case. The conidium (*a*, fig. 23) was sown in the morning about 8 o'clock, and remained almost unaltered for 24 hours; next day, at 2 P.M.—i.e., 30 hours after sowing—it had commenced to germinate (*b*), throwing out a delicate tube at either end; at about 10 P.M. (the same night) the stage *c* was reached, the germinal tube at the one end had grown to a short length only, and then its end had swollen up into a secondary conidium, taking the protoplasm from the rest. It will be noticed that the thin tail-like germinal tube at the other end of the conidium became empty, and that three septa appeared—one cutting off this empty tail-like tube, another dividing the main body of the conidium, and the third cutting off the successful germinal tube (as we may term it) from the now empty conidium. I mention these facts because it will be seen that these septa—usually three in number—constantly recur, and the tail-like unsuccessful appendage seems to be always formed and emptied as described. At 9 A.M. on the third day—i.e., 49 hours after sowing—the secondary conidium (fig. 23, *d*) had commenced to put forth a short lateral hypha, which by 2 P.M. (*e*) had grown out as a thin, feebly-coiled, and very delicate hypha, while a second similar hypha was forming above. These thin hyphæ grew a little longer, and then stopped (*f*).

In fig. 24 are shown other conidia germinating in the same way, under the same conditions; the slight variations in detail do not affect the general conformity to the above type. It frequently happens that two conidia "copulate" after they have formed the secondary conidia, or during the development of the latter (fig. 25). This often occurs when the conidia are numerous, but by no means always; the after-effects of such copulations, if they exist, do not manifest themselves clearly in the further fate of the secondary conidia; they appear to behave exactly as if no copulation had occurred.

There is a small point of some interest to be noticed here. It has already been stated that the cellulose wall of the conidium is exceedingly delicate; it results thence that when the conidium is deprived of its protoplasmic contents the remaining empty shell is barely visible, and easily overlooked, and the same is true of empty portions of hyphæ. It often happens, therefore, that such specimens as those in fig. 24 (*n* and *r*) are not at first sight quite intelligible, until more careful search results in the discovery that the empty conidia, &c., are still attached; in other cases, however, the remains of the conidia become destroyed (*e.g.*, by bacteria, &c.), and the delicate hyphæ containing the protoplasm persist alone. I have excellent reasons for believing that such hyphæ are not necessarily dead, and that the presence of certain fine hyphæ on the leaves is to be explained as above.

First, however, it will be advisable to see what occurs when the conidia are germinating in a drop of water on the leaf of the living *Ranunculus*, and where the increased supply of oxygen may be one of several causes for the fact stated above—that the conidia germinate more rapidly.

In figs. 26 and 27 are shown several specimens of germinating conidia, which had been sown in drops of dew on the living leaf, the plant being kept in a cool greenhouse under a glass bell-jar. It is at once noticeable that several of the conidia have proceeded at once to the development of the germinal hyphæ without the preliminary formation of the secondary conidia; the germinal tubes are thicker and stronger than is the case with sowings in pure water on glass. Here and there a case occurs (fig. 27) where the secondary conidium is interpolated as it were, but this at once proceeds to develop the germinal mycelium. Of course, the specimens figured are such as have not sent their hyphæ through a stoma; very many of them would do so about the second or third day after sowing, as shown in figs. 29 and 31.

It is now necessary to return to the mycelium in the intercellular spaces of the mesophyll of the leaf. It is observed in all cases where the white spots are fairly advanced, and wherever the conidia are developed in abundance on the exterior, that numerous spherical resting-spores exist among the closely weaved branching hyphæ. Thin sections and careful examination show further that these resting-spores are formed in the course of the hyphæ themselves as local dilations, or, more rarely perhaps, at the ends of branches which may be very short (figs. 9, 10, 17). It is possible to macerate or tease out specimens showing all the chief stages of develop-

ment. The resting-spore, at first simply a thin-walled dilation of the hypha, becomes separated by a septum, and its wall thickens gradually, bright granules and fat-like drops accumulating in the granular protoplasm. When quite mature, the protoplasmic contents assume a yellowish cast, but the thickened wall is only slightly yellow or colourless, perfectly smooth, and devoid of markings or mucilage, simply showing a slight tendency to stratification. It is not an uncommon event to meet with specimens like fig. 9, where one or two little branchlets arise close to the young spore and even appear closely applied to it (fig. 17); whether these are to be looked upon as representing degraded pollinodial branches, or whether they are merely of the nature of the haustorium-like branchlets referred to before, I cannot decide; it is quite certain that no fertilising tube is formed—the resting-spore arises purely asexually. The ripe spore exhibits a paler translucent spot in the centre, shining through the fatty and finely granular contents; it is perfectly easy to convince one's self of the attachment of the spore to the hypha in macerated specimens (figs. 10, 14, 19).

It is an obvious question: On what does the proof that the resting-spores and the conidia belong to the same fungus rest? The reply is simple and conclusive, though it has been by no means easy to obtain it. Putting aside the universal occurrence of the resting-spores in the white spots (fig. 1) as soon as they are well developed, and passing over the suggestive similarities to the "sporidia" of other Ustilaginæ, shown in the germination of the conidia (fig. 25), there are two series of observations which, together with these, place the connection between the spores beyond doubt.

In the first place the examination of very large numbers of careful sections has resulted in the obtaining of preparations like figs. 12-14, in which, although the anatomical continuity between a conidium and a resting-spore is not absolute, there can be no doubt as to the existence of that continuity. In fig. 14, the clearest case, the branch of the mycelium, passing up to the exterior, would end in a conidiophore, and it is attached to a branch bearing a resting-spore; and an examination of the other figure leads to the same conclusion: the difficulty of laying bare the hypha along its whole course is, of course, immense. The conclusion of the proof of continuity, however, is fully established by the production of the resting-spores from the conidia sown on the leaf.

I have already mentioned that when the white spots are at their best they are covered with the numerous conidia as with an impalpable chalky powder; the resting-spores do not come to the outside, and thus it is perfectly feasible, and even very easy, to obtain pure sowings of the conidia by lightly passing a camel-hair pencil over the spots on an undamaged leaf. I have paid a great deal of attention to this matter of pure sowings and pure culture, and most of the clean sowings on glass (*e.g.*, fig. 23) were obtained in this way: a clean, new camel-hair pencil was drawn lightly over a vigorous white spot, and a leaf infected in the manner described below, and then a glass slide was touched with the pencil, which still retained conidia; the purity of the last sowing was evidence of the purity of the infective sowing.

The method of infection is simple. The camel-hair pencil (or a clean needle), charged with conidia, is lightly placed for a moment in a drop of dew, or of distilled water, on a leaf of *Ranunculus Ficaria*. The sowing is then kept moist, either by means of a bell-jar placed over the plant, or by means of a damp-cell kept over the drop containing the sowing.

Precautions were taken to obtain the experimental plants from a distance, and from localities where no white spots were found on the leaves; moreover, I kept uninfected plants from the same neighbourhood in the same closed greenhouse, during the progress of the observations, as control plants. In all but one or two cases the infections succeeded perfectly, and in most cases the infective capacity (if I may so term it) of the conidia was most strikingly displayed.

It was perfectly easy to obtain such preparations as those figured at figs. 29 and 31 by stripping off the epidermis at the spot where the sowing had been made a few (12-24) hours previously; and similar preparations were obtainable any rainy day from the wet leaves in the ditch referred to above, especially when the leaf was first laid on water for a few hours. As the figures show, the conidia germinate normally, and at once proceed to push their germinal hyphæ through the wide orifices of the stomata; very often the germinal hypha makes coils, and it is usually at least sinuous. The unsuccessful tail-like hypha is developed at the opposite end of the conidium, as before; but the formation of the interpolated conidium is very rare—the germinal tube at once enters the stoma nearest it. Cf. figs. 23-27 and figs. 29 and 31.

When examining recently infected leaves, or young leaves from the damp ditch which were exposed to all the conditions necessary for infection, I often observed delicate little stretches of hyphæ lying on the cuticle, and looking like bits of a filamentous Schizomycete: two such bits are shown in fig. 29. Moreover, it was by no means uncommon occurrence to see similar filaments on the inside of a stoma closely applied to the walls of the guard-cells, and evidently making their way inwards. It seems not improbable that these isolated filaments are really pieces of the germinal tubes, which have been formed at some distance from the stomata, and have become detached by the decay of the exhausted portions of hyphæ or spores behind them. I have already shown that the protoplasm passes along into the ends of the delicate germinal tubes, leaving the empty and exhausted portions behind to die off (cf. fig. 24), and it is certainly not impossible that by this means these filaments can creep forward, so to speak, to distances greater than the tube full of protoplasm can reach—in fact, we may say the germinal tube creeps along by building its own ladder behind it.

Be this as it may, the leaves are easily infected by means of the conidia, and in nearly every case a pallid greenish-white spot was found on the infected leaf in from 13 to 19 days from the sowing: moreover, the spot was always confined to the area on which the sowing was made.

The following list of infections will serve to illustrate this. In each case three

leaves were infected—one leaf on each plant—and controlled as described. The date of sowing the conidia on the leaf is given in the second column, and the date on which the pallid spot was clearly visible in the third column: it should be noted that some difficulty occurs in deciding exactly when the pallid spot is visible, a difficulty which depends partly on the observer and partly on the hue of the leaf. The yellow spot of a Uredinous fungus is much easier to detect than these pallid greenish-white spots in their younger stages.

| Plant. | Date of sowing conidia | Date on which the spot appeared. | Number of days occupied in developing spot. |
|--------|------------------------|----------------------------------|---|
| | | | days. |
| A | May 13 . . . | June 1 | 19 |
| B | May 13 | May 31 | 18 |
| C | May 13 | June 1 | 19 |
| D | May 16 | June 2 | 17 |
| E | May 16 | Failed | |
| F | May 16 | Failed | |
| G | May 24 | June 7 | 14 |
| H | May 24 | June 7 | 14 |
| I | May 24 | Failed | |
| K | June 2 | June 21 | 19 |
| L | June 2 | June 19 | 17 |
| M | June 2 | June 20 | 18 |

Hence the periods given in the Table are only approximate—indeed, they could not be otherwise so long as we are ignorant of the exact period occupied by the conidium in germinating, and by the fungus in making its progress through the tissues.

And now arises the question—can we throw any light on the problem as to the relative ease with which a parasitic mycelium invades its host?

So far as it goes, the following evidence seems of some value. I found that those plants of *Ranunculus Ficaria* which grew in the shaded damp ditch were infected more easily than plants growing in open drier situations. The differences between these two kinds of plants, so far as the leaves are concerned, are chiefly as follows:—

The more shaded plants have much larger leaves with much longer petioles: the laminae are undoubtedly softer in texture, and brighter green in colour, the smaller tougher leaves of the plants in the open being of a dark and glossy green, especially above. These differences correspond to differences in minute structure: the shaded leaves have shorter, looser, palissade cells, and more intercellular spaces between them and in the large loose spongy parenchyma. Moreover, the stomata on the upper surface appear to be more numerous: on the lower surface the stomata seem to be larger, but I cannot say they are more numerous. The stomata have wider openings in the damp shaded plants, and the cell walls of all the parts are thinner and more watery; of course it may be assumed that there is more aqueous vapour in the intercellular spaces. Taking all these facts into consideration, I see no difficulty in explaining the differences in the times occupied in infections; and they also throw

light on the vexed question which has arisen around the unfortunate word "pre-disposition."

The plants in the ditch were certainly more apt to be diseased than others in the open, because the disease, once established, could spread like an epidemic under the conditions existing. I have other facts in other connections which bear out this, and I hope some time to be able to devote special and continuous attention to this question.

I do not propose to enlarge upon the subject of the resting-spores. After some months in the dormant condition they put forth promycelial tubes, from which sporidia are developed which seem to behave like the conidia described; the type of germination is like that of *Tilletia*.

DESCRIPTION OF THE FIGURES.

PLATE 10.

- Fig. 1. A leaf of *Ranunculus Ficaria* with the white disease-spots containing the parasite. Two of the white spots are turning ashen grey in the centre, and a still older spot at the margin had turned brown, and the rotted tissues then fell away. The chalky appearance of the younger spots is due to the conidia. There are spots on both sides of the leaf.
- Fig. 2. A stoma with the hyphæ of the *Entyloma* protruding—from a leaf laid 12 hours in water. To the right is one of the hyphæ about to form a conidium at the apex. The hyphæ are here very long, since they grow into the water. (ZEISS, E.)
- Fig. 3. Similar pencil of hyphæ protruding from a stoma, and bearing conidia. The preparation is taken fresh from a leaf growing in not very damp air, hence the shorter hyphæ and conidia. (ZEISS, E.)
- Fig. 4. Similar preparation—one of the conidiophores slightly branched. (ZEISS, E.)
- Fig. 5. Similar preparation from leaf in damp ditch, and taken in wet weather, showing the elongated form of the hyphæ and conidia. (ZEISS, J.)
- Fig. 6. Stoma with protruding conidiophores from the margin of a young spore. The fungus is still young. (ZEISS, E.)
- Fig. 7. A cell from the spongy mesophyll of *Ranunculus Ficaria*, with copiously branched hyphæ of *Entyloma ranunculi* closely applied to its walls. (ZEISS, E.)
- Fig. 8. Cells surrounding an intercellular space, with the mycelium of the *Entyloma* on and between the cells. (ZEISS, D.)

- Fig. 9. Portion of epidermis stripped off and examined from inside, showing branched mycelium of the *Entyloma* running between the underlying mesophyll cells (these are omitted for simplicity). Nuclei and plastidia are seen in the outlined epidermis cells. Resting-spores in various stages of development are being formed by the mycelium; most of these are intercalary, one is at the end of a short branchlet. Haustorium-like protuberances are frequently developed and some branches anastomose. (ZEISS, J.)

PLATE 11.

- Fig. 10. Similar preparation, the mycelium much branched. (ZEISS, J.)
- Fig. 11. Transverse section of a leaf of *R. Ficaria*, through a well-developed and active disease spot. The intercellular spaces are blocked up with mycelium and resting-spores; in the lacunæ below the orifices of the stomata the mycelium puts forth dense pencils of conidiophores. In the figure the stoma on the upper surface is cut longitudinally, that on the lower surface transversely and nearer one end. Conidiophores are also seen forcing their way between the epidermis cells of the upper side. The mycelium is all intercellular; wherever it appears otherwise, close examination shows that it is applied to the exterior of the thin walls. (ZEISS, D.)
- Fig. 12. Portion of extremely thin section through a disease spot, macerated and teased out. The two contiguous cells of the epidermis between which the hyphæ forced their way to the exterior have become separated. Two resting-spores among the hyphæ below. (ZEISS, J.)
- Fig. 13. Similarly teased preparation. The hyphæ are older and show the septa more clearly. (ZEISS, J.)
- Fig. 14. Portion of very thin section as before, hardened and examined in glycerine. It shows very clearly the passage out of the conidiophore in the middle lamella of two contiguous epidermis cells. The vertical branch to the right appears to have passed out similarly, but was cut; it is attached below to a branch bearing a resting-spore. (ZEISS, J.)
- Fig. 15. Lacuna between palissade cells from similar preparation; it is filled with resting-spores. (ZEISS, J.)

PLATE 12.

- Figs. 16-18. Mycelium with resting-spores teased out and isolated from macerated specimens. (ZEISS, D.)
- Figs. 19 and 20. Resting-spores in various stages approaching maturity. (ZEISS, J.)

- Fig. 21. Mature resting-spore. The contents are more finely granular and translucent; a portion of its mycelium is still attached. (ZEISS, J.)
- Fig. 22. A resting-spore commencing to germinate. (ZEISS, E.)
- Fig. 23. Germination of the conidium of *Entyloma ranunculi*. After lying twenty-four hours in water it swelled up slightly and presented the appearance drawn at *a*; six hours later, *i.e.* at two P.M., it had commenced to germinate (*b*); *c*, the same conidium at ten P.M.; *d*, at nine A.M. next day; the secondary conidium is now commencing to germinate. At two P.M. the stage *e* was reached, and soon afterwards (*f*) the growth had ceased, the two little germinal tubes having taken all the protoplasm. (ZEISS, E.)
- Fig. 24. Conidia germinating in water on glass as before. The empty conidia and other parts decay and disappear, and the still living portions are thus completely isolated. (ZEISS, E.)
- Fig. 25. Two germinating conidia which have copulated. This is a very common event where they lie close together. (ZEISS, E.)
- Fig. 26. Conidia germinating in drops of water on the leaf. The secondary conidia are not formed, as a rule, and the germinating hyphæ are stronger and branch more. (ZEISS, E.)

PLATE 13.

- Fig. 27. Similar preparation. (ZEISS, E.)
- Fig. 28. Conidia attached to branched conidiophore (below), which had been allowed to grow out in water; and a germinating conidium; both more highly magnified. (ZEISS, J.)
- Fig. 29. Portion of epidermis of *R. Ficaria* with conidia of *E. ranunculi* germinating on it. Germinal hyphæ are entering the orifices of the stomata; two pieces of germinal hyphæ (?) are lying on the epidermis cells. Inside the latter are nuclei and plastidia. (ZEISS, E.)
- Fig. 30. Portion of mycelium allowed to grow out into water; the protoplasm aggregates in certain cells and branches, and the septa are very distinct in the empty branches. (ZEISS, E.)
- Fig. 31. Stoma with two germinal hyphæ entering its orifice; seen from within. The spores are visible through the epidermis. (ZEISS, E.)
- Fig. 32. Stoma seen from within. On its walls are thin branched hyphæ of unknown origin, but which may possibly be isolated pieces of germinal tube. (ZEISS, E.)

IX. *Researches on the Structure, Organization, and Classification of the Fossil Reptilia*.*—I. On *Protorosaurus Speneri* (VON MEYER).

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[PLATES 14–16.]

Protorosaurus Speneri, one of the earliest known fossil reptiles, has been already studied and described by Baron CUVIER, HERMANN VON MEYER, Sir RICHARD OWEN, and Professor HUXLEY. Occurring in the Kupferschiefer, and therefore of Primary age, the exact determination of its structure and affinities has become of some interest in relation to the great development of Reptilian life which characterises the succeeding Triassic period.

The most interesting example of *Protorosaurus* is that originally obtained by SPENER, which he described and figured in 1710, and regarded as the remains of a Crocodile.† His view was confirmed by LINK. But KUNDMANN of Breslau in 1737 interpreted the remains as those of a new type of large-headed fossil-lizard. This conclusion was substantially adopted by CUVIER, who in 1808 made the animal universally known as the fossil Monitor of Thuringia.‡ CUVIER had never seen a specimen; and was dependent upon the figures published by SPENER, LINK, and SWEDENBORG, and a drawing, which he published, of a specimen preserved in the Royal Museum at Berlin. He remarks that the head is not without resemblance to that of the Nilotic Crocodile, and, as SPENER only knew drawings of the exterior of

* Some time ago the Royal Society did me the honour to place at my disposal grants from the Government Grant Fund, for the investigation of the Fossil Reptilia. They enabled me to make studies and preliminary descriptions of a large mass of materials in Continental and English collections. Some of these, which were chiefly of geological interest, were laid before the Geological Society. Others needed further work before they could be used to elucidate the structure, organization, and classification of the Fossil Reptilia. The general results to which the researches have led are necessarily connected with the detailed evidence on which they rest; and I now propose to submit to the Royal Society any account of such genera and ordinal groups as fall within this field of work, as well as discussions of the distinctive osteological organization which some orders have in common, before summarising the classification.

† 'Miscellanea Berolinensia,' Berolini, 1710, T. 1, p. 99. "Disquisitio de Crocodilo in Lapide, &c.," figs. 24, 25.

‡ 'Annales du Muséum,' T. 12, p. 79, Plate 10.

the Crocodile, his identification was excusable. CUVIER goes on to argue that the number of teeth in the lower jaw of a Crocodile is at fewest fifteen, while in the upper jaw there would be seventeen or eighteen extending back to the middle of the orbit, whereas the fossil has only eleven teeth, which reach back to the anterior angle of the orbit. On this evidence the skull is interpreted as that of a Lacertilian, allied to the Monitor. The author goes on to show that other parts of the skeleton confirm the inference from the skull. Thus the hind limb has five digits, with the number of phalanges in them successively 2, 3, 4, 5, 3, which agrees with the Monitor. The correspondence extends to the larger bones of the extremities. CUVIER only detected two differences of specific value: first, the spinous processes of the dorsal vertebræ are much more elevated than in Monitors; and secondly, the foreleg is relatively longer in proportion to the femur and the foot. It is unnecessary to offer any detailed discussion of this interpretation, for the figure now given, when compared with SPENER's figure, shows that CUVIER had not the evidence fairly before him.

VON MEYER also studied the published figures, and came to the conclusion that the fossil was neither a Crocodile nor a Monitor; but that it was an extinct type which differed by remarkable modifications and peculiarities from the Saurian group. In consequence he founded the genus *Protorosaurus* in 1830, and described the species as *Protorosaurus Speneri* in 1832.* CUVIER's influence, however, continued to govern the views held as to the affinities of this animal, although VON MEYER's name was adopted in OWEN's 'Odontography.' Eventually VON MEYER, finding in various museums twenty-one specimens which appeared to him referable to *Protorosaurus*, made these fossils the subject of an elaborate monograph with nine folio plates, published in 1856.† Nearly all these specimens were studied and measured by the author. But unfortunately the type, which passed into the collection of JOHN HUNTER, was unknown to him, and he reproduces in outline SPENER's unsatisfactory figure of 1710. Yet such was VON MEYER's confidence in the figure that he supposes the soft parts about the mouth to be preserved. Nothing of value, therefore, is contributed to knowledge of the skull. The whole of the specimens are referred with some doubt to one species; and a detailed anatomical description is given of the several regions of the skeleton. The neck is suggestive of the vertebræ of Ornithosaurs and of the Giraffe, but is not compared with that of a Bird because the number of cervical vertebræ recalls that of the Crocodile. The dorsal vertebræ are more numerous than those of the Crocodile, but their shape differs from that seen in all living Saurians [as then known]. The ribs on the whole were Lacertilian. The absence of lumbar vertebræ was regarded as conclusive against affinities with Monitors. The sacral vertebræ in the several examples are considered to number two, three, or four. The tail vertebræ are distinctive in having the neural spine divided. In the shoulder-girdle some resemblances are seen to Archægosaurus. No important

* 'Palæologica,' 1832, pp. 109, 208.

† 'Fauna der Vorwelt,' "Saurier aus dem Kupferschiefer der Zechstein-Formation."

conclusions are drawn from the larger bones of the limbs. And the hand and foot both show Lacertilian characters. There is no dermal skeleton. This description is the basis of most accounts of the animal which have been published. I find no difference from VON MEYER possible except perhaps as to the reference of the remains to one species, and as to the absence of a dermal skeleton.

Sir RICHARD OWEN first noticed *Protorosaurus Speneri* in his 'Odontography,'* and subsequently, in his 'Catalogue of the Fossil Reptiles and Fishes in the Royal College of Surgeons' (1854, p. 80), mentions that the specimen there preserved is SPENER's type, which passed into the collection of Dr. JOHN WOODWARD, and was purchased by JOHN HUNTER at the sale of Humphrey's Museum.† In Sir RICHARD OWEN's 'Palæontology'‡ it is stated that the head equals one-third the length of the neck and trunk, and resembles in shape a long, slender, obtusely-pointed cone. It has strong straight jaws, armed with sub-slender, sub-equal, straight, conical, sharp-pointed teeth; about eighteen on each side of the upper, and sixteen on each side of the lower jaw, implanted in a single close-set series of sockets. After describing the remainder of the skeleton, it is remarked: "Of existing Reptiles the largest carnivorous Varanian Monitors (e.g., *Varanus*, *Hydrosaurus*) offer most resemblance to the *Protorosaurus*, which had evidently the same powers of progression, as well on land as in the water. But this oldest known Lizard presented a more powerful and complex framework. The neck is longer and stronger, the vertebræ rivalling in proportion those of Pterodactyles; the head is relatively larger and with more firmly fixed teeth; the dorsal spines are loftier and larger than in modern Monitors; the larger sacrum accords with the relatively larger and stronger hind limbs. The more numerous diverging processes for the attachment of the tail muscles bespeak the more vigorous actions of that part. All the vertebral bodies have sub-concave articular ends, and it may be concluded from the length and strength of the tail, from the peculiar provision for muscular attachments in that part, and from the proportions of the hind limbs that the *Protorosaurus* was of aquatic habits, and that the strength of its neck and head, and the sharpness of its teeth, enabled it to seize and overcome the struggles of the active fishes of the waters which deposited the old Thuringian copper slates." This animal was referred provisionally, and with doubt, to the order Thecodontia. But some evidence has since been adduced by Professor HUXLEY to show that *Thecodontosaurus* and *Palæosaurus* may be classed with Dinosaurs; so that, if the Thecodontia should be sustained as a group distinct from the Parasuchia, which appears to be synonymous, the suggested affinities would indicate that *Protorosaurus*, although written of as a Lizard, was regarded as approximating to Dinosaurs and their Crocodilian allies. I find myself, however, differing from Sir RICHARD OWEN as to the condition of the teeth, for I can detect no conclusive

* Vol. 1, p. 269.

† 'Descrip. Cat. Fossil Rept. and Pisces,' 1854, p. 80.

‡ P. 280, 2nd ed., 1861.

evidence that any were contained in sockets. And, if so, the evidence disappears which would refer the animal to the Thecodontia. The other characteristics mentioned are essentially a summary of the views of CUVIER and VON MEYER, unsupported by new evidence.

Professor HUXLEY discussed this animal in his 'Anatomy of Vertebrated Animals,'* classing it with Lizards, in a position intermediate between the fossil group Homœosauria and the Platynota, which comprises the Old World Monitors. VON MEYER's suggestion that it is the type of a new group is adopted, and the group is named Protorosauria. The skull is said to be of moderate size, preserved in one specimen only; and in that it is in such an imperfect condition that the details of its structure cannot be made out. The teeth, however, are nearly straight, conical, and sharply pointed, and seem to have been implanted in distinct sockets, though there may be some doubt on this point. The tail is long and slender, and the limbs well developed, as in the existing Monitors. In the abdominal region numerous short and filiform bones appear to represent and correspond with the abdominal ribs of Plesiosauria and Crocodilia. Beyond the middle of the tail the spinous processes bifurcate, so that each vertebra seems to have two spinous processes, a peculiarity unknown in other Lacertilia. The large chevron bones are articulated between the bodies of the caudal vertebræ, as in Crocodilia, but also as in some Lacertilia, such as the *Geckos*. In the pes the number of phalanges is characteristically Lacertilian, and so is the form of the metatarsals. The tarsal structure is compared with that of the *Geckos*. I find the skull crushed and badly preserved, but perfectly intelligible.

A specimen from Durham, described by Messrs. HANCOCK AND HOWE, adds nothing to the scientific history of the type, beyond its presence in a British Permian deposit.

In these several studies there are substantially only two interpretations of *Protorosaurus*; first, CUVIER and HUXLEY class it unreservedly with Lizards; secondly, VON MEYER and OWEN refer it to a new Reptilian type. VON MEYER affirmed that it is neither Lizard nor Crocodile; but saw in it resemblances to those animals, as well as to *Archægosaurus* and Pterodactyles. The difficulty in harmonising these different views has been partly in want of knowledge of the skull.

Professor CHARLES STEWART, Conservator of the College of Surgeons Museum, having recently rearranged the Reptilia, and placed SPENER's fossil in an accessible position, I have been able to make some notice of its structure. And I have to thank the President and Council of the College for permission to obtain drawings of the remains; and to thank Professor STEWART and Dr. GARSON for facilities afforded me in making the following description of the type of *Protorosaurus Speneri*.

PART II.

The Specimen in the Museum of the Royal College of Surgeons.

As figured by SPENER, the skull is represented as having a blunt conical snout, which overlaps parts of some vertebræ, so as to terminate at the junction of the centrum and neural arch. The extremity of the jaw for a length of 12 or 13 millims. has been destroyed since that figure was drawn (Plate 14), so that, though the skull, as preserved, is 7 centims. long, it may originally have been $1\frac{1}{2}$ centims. longer. This destruction of the anterior end of the jaw makes it impossible to determine whether the anterior nares occupied a terminal position as in Crocodiles, or whether they are to be sought in the small ant-orbital vacuities, which we shall find situate, like the nares, in *Ichthyosaurus*. This uncertainty affects the interpretation of the bone which carries the teeth. It may be either pre-maxillary or maxillary; but can only be maxillary on the hypothesis that the pre-maxillary bones are lost. But SPENER's figure gives no indication of the nares having been terminal, and so far is evidence against that condition; and the position of the nares must be inferred from their condition in the animal types to which the fossil may prove to approximate.

The skull is crushed and flattened obliquely, so as to display its left side, together with the roof bones of the head. It is displaced from connection with the vertebral column, and its hinder lateral region is covered up by the anterior cervical vertebræ, which obliterate the bones which would demonstrate the affinities of the animal.

The cranial bones are all remarkably dense and thin, in harmony with the large medullary cavities and thin walls of the limb bones; and this osseous condition approximates to that which characterises the bones of Ornithosaurs and Birds. Some approach to this condition is seen in the limb bones of Lacertilia, and in Crocodilia and Dinosauria, though some American fossils referred to the Dinosauria, such as *Megadactylus polyzelus* (HITCHCOCK),* have the walls of the limb bones thinner. The solid character of the articular ends of bones in *Protosaurus*, however, would indicate a method of ossification by conical terminal epiphyses descending into the shaft, like that which characterises the Batrachia, Plesiosauria, and certain Chelonia; so that the evidence of affinities must be fully stated before any conclusions can be based upon the thinness of the cranial bones.

The brain-cavity.—The region of the brain is seen to be very narrow from side to side posteriorly towards the occiput, and to widen transversely as it extends forward towards the orbits. Portions of the parietal and frontal bones are lost, and their removal shows that the cerebral hemispheres were well developed. They are convex in length, broad, defined anteriorly by a groove in the matrix, and rounded anteriorly as though the brain case were closed anteriorly by bone. The lateral compression of the

* COPE, 'Trans. Am. Phil. Soc.,' vol. xiv., Plate 13, p. 122A.

part of the parietal region which is posterior to the cerebral hemispheres shows, I believe, that the cerebellum was relatively narrow and thrust downward in the way seen in Birds, Ornithosaurs, and Dinosaurs. The length of the cerebral region is about 15 millims, so that the head would be $5\frac{2}{3}$ times as long as the brain. The cerebrum may occupy 11 to 12 millims. in length; its width is less evident, but appears to have been about 7 millims. The hemispheres were high, and flattened at the sides, so that on the whole, in so far as the brain differed from that of an Ornithosaurian, it appears to have approximated to that of a Dinosaur. There appears to be a small parietal foramen placed far back, and in advance of it there is a slight oblong inflation of the cast of the cerebral cavity. I describe in succession the median roof bones of the head.

Supra-occipital.—The supra-occipital region of the skull is imperfectly exposed, since only the portion is seen which lies above the occipital foramen. It looks obliquely upward and backward. It is defined anteriorly by the occipital crest. This crest is in two lateral portions, which meet mesially at about a right angle, and diverge outward and backward. The posterior surface of the bone is divided into two shallow, lateral, concave areas by a slight sharp median inclined ridge.

The parietal bones.—The parietal region is greatly compressed from side to side in its hinder part, so as to rise into a short sharp parietal crest (now broken away), which made the sides of the bone concave from the occipital crest forward. The length of this compressed area is only a few millims. On it the small ovate parietal foramen appears to be placed. In front the bone is lost, but I think the horizontally flattened state of the frontal bone anteriorly, and the comparatively flattened state of the mould of the cerebral hemispheres, together with the thinness of the bones, justifies a belief that the parietal bones became flattened superiorly as they widened and extended forward, and that the parietal crest was moderately elevated. There were two parietal bones, and the median longitudinal suture between them is seen as an elevated line on the mould beneath, where the bones are lost. The transverse suture between the parietal and frontal bones is at a distance of about 12 millims. in advance of the median angle of the occipital crest. This suture has a transverse saw-like edge, and admits the median extremity of the parietal bones to extend slightly forward between the hinder margin of the frontal bones.

The frontal bones.—The frontal bones are double, being united by a median suture. They exhibit an oblong surface, which was flattened horizontally. Their anterior extremities extend forward between the nasal bones in a V-shape, while the lateral parts of this suture diverge forward and outward. Posteriorly, the outer corner of the bone on one side is notched out by what appears to be the temporal foss, and, although the temporal arcade is not preserved, it may have extended backward from the narrow post-frontal process external to the notch in the manner seen in Ornithosaurs, Dinosaurs, Nothosaurs, or Anomodonts. The lateral borders of the frontal bones are concave, 15 millims. long, and are superior margins of the orbits. They are slightly

raised and transversely roughened. The least transverse measurement across the frontal bones at the middle of the orbital concavity was 11 millims. In the median line, behind the middle of the orbits, the frontal bones form a slight longitudinal median ridge, anterior to which a wide shallow median concavity extends forward, and is prolonged down the upper surface of the nasal bones.

Prefrontal and lachrymal bones.—There appears to be a slight channel above the anterior border of the orbit, which is increased by a slight displacement of the prefrontal bone. This bone forms the anterior border of the orbit. It widens as it extends forward and downward from the middle of the orbital border in the frontal bone to the dentigerous bone, which for the present may be premaxillary or maxillary. This bone is 15 millims. long by about a centim. wide where widest, in its lower third. It extends under the frontal bone above, and overlaps the dentigerous bone below. A suture divides it transversely, so the lachrymal bone is present as a separate ossification. In front of the lachrymal bone is a notch, which also indents the upper hinder part of the bone which carries most of the teeth. This foramen is led up to by a longitudinal channel in the dentigerous bone. The nasal bones would have reached the superior border of this foramen. Hence it is evidently an ant-orbital vacuity, but whether it is comparable to the ant-orbital vacuity of Teleosaurs, Dinosaurs, Ornithosaurs, and Birds, or to the ant-orbital vacuity of Ichthyosaurs, which is similarly placed, and forms on each side of the head the anterior nares, depends upon the interpretation of the bones which form its anterior borders.

The nasal bones.—The nasal bones roof over the head in front of the orbits, and are united by suture with the frontal bones behind. They are imperfect anteriorly, but as preserved are 3 centims. long. They are united by a median straight longitudinal suture, and form a shallow longitudinal concavity extending forward on the snout. They have a transverse width of 12 or 13 millims. posteriorly, and narrow anteriorly to a width of 4 or 5 millims. at the anterior fracture. Laterally, each bone makes an angular bend downward, so as to overlap and make a squamous union with the long dentigerous bone which runs parallel to it and forms the toothed margin of the jaw.

The question whether that bone is premaxillary or maxillary may now be examined. If the converging borders of the nasal bones were prolonged anteriorly, they would terminate one centim. in advance of the fracture, or half a centim. from the extremity of the jaw. Hence it is probable that if the nares were terminal they were small, though not smaller than in some Lizards. The large nasal bones, however, are not Lacertilian, and find no parallel so close as may be seen in *Ichthyosaurus*. And then the dentigerous bone would closely resemble the premaxillary bone in those Ichthyosaurs in which the nasal bones extend to near the end of the snout. A corresponding elongation of both nasal and maxillary bones is seen in Crocodiles; but the anterior groove, which in *Protorosaurus* runs up to the ant-orbital vacuity, is similar to that seen in Ornithosaurs and Birds; and this leads me to regard the ant-

orbital vacuity as probably nasal, and consequently the dentigerous bone as probably premaxillary, though the morphological data for the identification are confessedly slender.

The premaxillary bones.—This bone resembles the same element in *Ichthyosaurus* in steadily augmenting in depth as it extends backward. Its upper hinder margin is notched out by the vacuity which I am disposed to regard as nasal. The form of the bone is a long triangle with its narrow base towards the orbit. Its depth posteriorly, as exposed by the removal of the covering nasal bone, is about one centim. Its length as preserved is between 3 and 5 centims. There is a longitudinal groove above the bases of the teeth, like that seen in *Belodon*, and which indents the *Ichthyosaurian* jaw parallel with the base of the dental groove. The rough convex surface of bone between this groove and the alveolar border has been removed along its length, apparently to expose cavities like sockets which may have been for successional teeth, of which 18 are visible. Although these pits existed beneath the teeth which were in use, there is no evidence that those teeth were in sockets. The teeth were manifestly ankylosed to the jaw as in *Lybyrinthodonts* and some lizards. A horizontal plate appears to have divided the base of the tooth from the quadrate cavity beneath. One tooth appears to be in one of these sockets. The teeth were closely set, but are nearly all wanting, and only indicated by the infra-dental cavities and by impressions of the crowns. There is no trace of successional tooth in any other of these infra-dental spaces. They are uniform in size and depth, and in most cases, but not always, immediately beneath the crowns. They are not circumstanced like cavities for successional teeth so far as these are known, and are apparently interior in position to the teeth on the alveolar margin. The crowns of the teeth appear to have been smooth, conical, pointed, with the base circular. One of the longest, in front, measures 5 millims. from the point to its ankylosis with the jaw, and about 7 millims. to the bottom of the infra-dental cavity.

The maxillary bone.—The posterior part of the dentigerous border may be a separate bone, but if so the suture which defines the maxillary bone is not clearly made out. It probably is in front of the last two infra-dental cavities, above a depression which indicates a squamous overlap upon the premaxillary bone. As it extends backward below the orbit, three or four slender pointed teeth are seen to extend from it, but more may be hidden in the matrix. The bone terminates backward in an oblique suture which is below the middle of the orbit, and therefore presumably indicates the jugal bone, which is imperfectly exposed and apparently displaced downward.

Above the maxillary region the cervical vertebræ lie over the orbit and the back of the head. The violence which separated the vertebral column disengaged the lower jaw and separated its elements, and displaced the quadrate bone and bones of the palate, which lie scattered between the head and the lower jaw, not entirely free from matrix.

The sclerotic circle.—Below the orbit a structure exists which closely resembles the sclerotic armature of a Bird (Plate 15), which, when complete, may have approached a diameter of 2 centims. A circle of this size might have been contained in the orbit. It is inflated in the middle part, in the centre of which appears to be a smooth space of matrix; externally its border is concave. It appears to be formed of radiating thin plates in close contact; but the state of preservation does not admit of detailed description or absolute identification, for the mass may possibly be dermal armour.

The bones of the palate.—The bones of the palate are scattered. Their identification rests upon, first, the forms of the bones; secondly, their consecutive positions; and thirdly, the fact that the vomer, palatine, and pterygoid all carry minute teeth; while there can be no suspicion that these elements belong to the lower jaw, since the lower jaw is preserved.

Vomer.—Both vomerine bones are indicated, and both are partly imbedded in the matrix. They were very slender, 3 centims. long, and about 2 millims. wide where widest proximally. They carried minute teeth, densely placed along the margin. The crowns are enamelled, enlarged and pointed, with lanceolate form.

Palatine bone.—The palatine bone is a long triangle, notched out on the inner anterior margin for the reception of the vomer. The bone is 3.2 centims. long, and 8 millims. wide posteriorly, tapering away in front. The external border is straight. The posterior border is straight and truncated, but rounds into the inner side, which is depressed where it received the anterior limb of the pterygoid. The surface of the bone is rather convex till it becomes channelled with the groove which leads forward to the notch for the vomer. The vomer probably extended along much of its inner margin (fig. 1, p. 19). Along the external margin of the palatine bone a row of teeth extended. They were rather larger than those on the vomer, though only one or two are preserved.

The pterygoid bone.—The outline of the pterygoid bone is not easily traced. The bone is in accidental contact with the palatine bone, and probably in natural union with the quadrate bone. It is stronger than the palatine bone, short and broad posteriorly, sending a long sharp process forward which I regard as extending interior to the palatine bone (fig. 1, p. 19). This process or bone is 1.6 centim. long, 3 millims. wide at the base, and tapers to a point. It carries a few minute teeth, some of which appear to be barbed. The interpretation of the posterior part of the bone is more difficult, because the bone originally extended in more than one plane; and it is impossible to determine with certainty whether the expanded transversely oblong plate which is in contact with the quadrate bone is in natural union. I assume the connection to indicate the true relation of the bone. Then it follows that the oblong truncated expansion of the bone which is at present in contact with the palatine must be internal, and either have articulated with the basi-sphenoid, as in Lizards and Anomodonts, or else with the corresponding surface of the other pterygoid bone, as in Dinosaurs. Then there would be no lateral plate for the internal pterygoid muscle

such as is seen in Crocodiles and Lizards, but the great oblong plate which extends outward to the quadrate bone must have been attached along much of the length of that bone.

The quadrate bone.—Posterior to the pterygoid bone is a much stronger bone, imperfect at both ends, which I regard as the quadrate bone. As preserved, it is 1.4 centim. long. It is somewhat compressed, constricted in the middle, and expands proximally to a width of 4 millims. It has an internal expansion which is not fully seen, which is wide, thin, and oblique, and appears to be the pterygoid process. The surfaces of the quadrate bone are smooth, and concave in length in every direction in which exposed.

The lower jaw.—The lower jaw has its constituent bones displaced. As preserved, it is about 9 centims. long, and measures 8.5 centims. from the articulation for the quadrate bone to the extremity of the dentary bone. There is no indication of a coronoid process. It is long and narrow, increasing a little in depth as it extends backward, but becomes less deep again towards the posterior articulation. The outline is straight along the dentary border, and slightly convex below. Both dentary bones are present, and show that they had only a narrow union at the extremity of the jaw, and were not anchylosed together. The extreme length of the dentary bone was probably about 6.5 centims. The angular and surangular were both elongated bones. The splenial bone appears to have lapped along the inner side of the jaw and extended forward to near the extremity of the dentary bone. The articular bone is lunate, 8 millims. long; not unlike this bone in the Crocodile, with a transverse concave articulation. The bone, though now exposed, was probably imbedded. Twenty-seven teeth can be counted apparently anchylosed to the dentary bone, extending along a border of more than 4 centims. Other teeth may have been present further back.

Hyoid bones.—Between the articular end of the lower jaw and the displaced quadrate and pterygoid bones are slender, delicate, straight, cylindrical bones, very imperfectly displayed, which are jointed. Their slenderness and position are suggestive of the hyoid elements. The length exposed is 2.2 centims. The structure apparently consists of a rod measuring 1.3 centim. and two short joints of about 2 millims. each. The terminal joint is conical.

The vertebral column.—The vertebræ extend in a continuous curve, with the neck bent round so as almost to meet the sacrum; beyond which the tail extends, at first gently curved, and then almost straight. About 59 centims. of the vertebral column are preserved, but a portion of the tail, of unknown length, is lost.

The cervical vertebræ.—The cervical vertebræ are conspicuously elongated (Plate 14, 2-7). Six are preserved in connected sequence. Measured round the curve, they have an aggregate length of 13 centims. The Atlas does not appear to be preserved, or, if preserved, is broken, and the fragment out of position and imbedded in matrix. As the first of the series is the short Atlas, this animal appears to have had seven cervical vertebræ. Being in close union by means of the several articular processes,

the forms of the articular ends of the centrum are imperfectly seen, but the condition displayed by the third vertebra of the series (Plate 14, 4) appears to show that the intercentral articulation in that vertebra, at least, was opisthocœlous.

The first vertebra, which is very imperfectly preserved, has the centrum 1.9 c.m. long. The second is of the same length. The third vertebra is the longest, and measures 2.5 centims. The fourth is about a millim. shorter. The fifth measures 2 centims.; and the sixth, which is badly preserved, is about 1.8 centim. long.

In relative elongation as compared with dorsal vertebræ, these cervical vertebræ show a character which is most closely paralleled among Ornithosaurs, but is also met with in various existing Birds and Mammals. Some Chelonians have the cervical vertebræ of a similarly long form; and in the fossil the zygapophyses have a development which is scarcely equalled among Chelonians. The strong, broad, elevated neural spine is distinctive.

The external layer of bony tissue in these vertebræ appears to be as thin as in an Ornithosaur, or a Dinosaur like *Calurus*, as though the centrum were occupied by an air-cell. But, although there is a small foramen in the middle of the side, in a position which might coincide with the junction of the centrum and the neural arch, it is scarcely larger than the ordinary nutritive foramen, common in such a position, and gives no indication of a pneumatic function. The forms of the articular surfaces make the inferences probable that the neck was carried or capable of being carried in a vertical position, as in the Galapagos Tortoises. Only the side of the vertebræ is exposed.

In the third vertebra (Plate 14, 4) the centrum is marked with three narrow, sharp, sub-parallel ridges which extend in curves between the anterior and posterior articulations; they are but little elevated, and give a channelled aspect to the side of the centrum. The anterior articular ball of the centrum appears to be well ossified; it is about 6 millims. deep, and hangs obliquely forward. There is a less obliquity in the posterior cup, which is somewhat deeper, and is defined by a sharp margin. The neural arch extends along the centrum. The anterior and posterior borders of the neurapophysial lamina which margins the intervertebral neural foramina are concave from above downward, convex from within outward. The antero-posterior distance between them is 2 centims, and the arch, as usual, ascends from the centrum close to its anterior end, just over the anterior articulation. From the upper side of the neural canal the prezygapophysis extends forward and upward. It is 8 millims. long and 2 millims. wide, and its upper surface is 1 centim. above the base of the centrum. Immediately behind it is a strong ridge or slight transverse process which connects with the posterior zygapophysial process. Its transverse extension outward is broken away. The extreme measurement between the extremities of the zygapophyses is 3.1 centims. The neural spine is sub-quadrate, compressed from side to side, rising about 9 millims. above the interzygapophysial ridge. The upper border is truncate, slightly rounded from the post-zygapophysis behind, as it extends forward and upward. The front border of the neural spine leans a little forward. In the fourth vertebra the

neural spine has its anterior border more vertical, but in both the posterior border is inclined obliquely backward, so that the spine becomes less high towards its posterior extremity.

Slender cervical ribs are attached to the anterior extremities of the sides of the centrum. The ribs are straight, slender, pointed behind, are as long as the vertebrae to which they run parallel, and have thickened heads for attachment.

Near these cervical ribs are several slender, long, tendinous ossifications, such as are often met with in the vertebral column in Birds and Mammals, and are common in the caudal region of the Bernissart Dinosaurs.

Dorsal vertebrae.—There appear to have been sixteen dorsal vertebrae in the 19 centims. between the neck and sacrum. None of these vertebrae are so preserved as to be worth description. They give little information about the centrum, but show that the neural spines were 9 millims. high in the anterior vertebrae, vertical, 6 millims. wide, with the anterior and posterior margins parallel, and the truncated superior outline convex. The ribs appear to have been attached to short transverse processes or tubercles given off from the neural arch. The centra appear to be bi-concave or flattened at the ends.

The ribs are strong, curved, and compressed from front to back.

Sacrum.—The sacrum appears to have included two vertebrae. They are short, and had well-developed transverse processes, 12 millims. long, which expanded externally. But the bones are too badly preserved to demonstrate any other point of structure.

Caudal vertebrae.—Twenty-three caudal vertebrae are preserved. Each centrum is 1 centim. long. The body of the centrum is compressed from side to side, and rounded on the base. At about the level of the neuro-central suture transverse processes are developed in the earlier part of the series. Then the neural arch rises, and develops short zygapophyses on the level of the summit of the neural canal, and forms a short platform from which the high vertical neural spine rises. The measurement from the base of the centrum to the neural platform in the earlier caudal vertebrae is 1 centim.; and the height of the neural spine above the platform is 1.2 centim. The caudal vertebrae after the first two have long spatulate chevron bones, which are directed obliquely backward. They are at first very long, and then become gradually shorter. With their development the neural spine becomes constricted at its base and wider at the summit, so that it gradually assumes a wedge-like form. At about the 14th caudal vertebra, 11 or 12 centims. behind the sacrum, the summit of the neural arch is notched. The spine after this continues to decrease in height as the notch increases in depth, until after about six vertebrae the neural spine is completely divided into anterior and posterior parts, which have the spines obliquely directed backward and forward, with an increasing interval between them. As they are followed backward, the vertebrae diminish in all dimensions except length. And the neural spine decreases in height, while the transverse process, which at first is

strongly marked, soon sinks into insignificance, and appears to be lost before the neural spine becomes divided.

The hind limb.—There is no bone of the pelvis preserved.

The femur and bones of leg lie in natural position, with the head of the femur towards a transverse process of a sacral vertebra. That process (Plate 14, *sa*), as in other animals, is wedge-shaped, 11 millims. in transverse extension, and 9 millims. in antero-posterior extension on its external limit, as preserved. But it is imperfect, and may have been wider, and may not have been so much constricted where it joined the centrum as the present state of the fossil would indicate. These processes indicate a strong pelvis. Little of the strong straight femur now remains except the crushed impression of its outline, in which some fragments of bone still adhere (Plate 14, *f*). The length of the impression is 7.1 centims. The proximal articular surface does not appear to have been in quite the same plane as the distal surface. The proximal end is 1.6 centim. wide, with the head convex and directed laterally, but with a broad process or trochanter 6 millims. wide, which extends a few millims. proximally beyond the external border of the articular surface. If this fragment of bone is correctly interpreted, the articulation presents a condition which is only paralleled among Birds, Ornithosaurs, and Dinosaurs, though the proximal trochanter is less developed in Dinosaurs than in this fossil. The sides of the bone approximate so that the transverse measurement in the middle of the shaft is 8 millims., which width is preserved without appreciable diminution to the distal end of the straight shaft. The distal articulation is rounded from above downward, and slightly thickened on the posterior condylar aspect, as in a Lizard, Bird, or Ornithosaur. The bone was hollow, with a very large cylindrical cavity in the shaft, quite as much developed as in Wealden Ornithosaurs and many Birds, and with the bony tissue quite as dense, though Lizards make an approximation in both respects.

The tibia and fibula are imperfect distally, and only 4.7 centims. of the bones are preserved (Plate 14, *t, fi*). What remains of the bony tissue shows that the bone was thin in the middle of the shaft, with a large medullary cavity. The proximal end of the tibia is truncated, with rounded margins. Its transverse width is fully 11 millims., while the transverse measurement in the middle of the shaft is only about 3 millims. This proximal expansion is partly due to a general Bird-like or Dinosaurian massiveness of the proximal end of the bone, and partly to the development of a not inconsiderable cnemial crest, which speedily subsides distally, but forms a ledge against which the fibula rests. The proximal end of the bone is more solid than in existing Lizards. Where fractured, the bone is enlarging distally. The fibula is a more slender bone, with a slight sigmoid curve, nearly uniform in width, being 3 millims. wide in the proximal half, and a little narrower distally.

The foot.—In the region between the cervical and dorsal vertebræ remains of an extremity of a limb are displayed. A metapodial bone is 2.1 centims. long; extremities of other bones of a like character are exposed. Extending beyond them

are the impressions of four digits, which successively augment in length. They show the increasing number of bones, indicated by the formula 2.3.4.5. All the articular surfaces of the phalanges are perfectly ossified, and they are shaped as in Dinosaurs, but an approach to this perfect ossification is seen in the *Protosaurops* and other fossils. The terminal phalanges of the digits are in the form of claws, curved and pointed, and compressed from side to side.

Armature of the skin.—In the region of the early dorsal vertebrae a fragment is exposed of a very thin plate of bone which was at least 3 centims. long. It is made up of a number of minute oblong bones, each 1 millim. wide, sutured together into a shield across which a slight longitudinal keel runs. This plate I regard as a piece of dermal armour. (Plate 15, fig. 11.)

PART III.

Comparison between the Type in the Royal College of Surgeons Museum and other Specimens referred to Protosaurus Speneri by VON MEYER.

Before an attempt is made to explain the structure of this type of animal, it is necessary, on account of its imperfect preservation, to discuss its relations with the specimens figured by VON MEYER. That great anatomist was disposed to regard the differences between the fossils as due to age and resultant differences in ossification, though he did not decide absolutely on the specific identity of the whole of the materials. Unless the specimens could be brought together, it would be difficult to determine their relations so as to assign its systematic place to each, for the animals have so much in common, and the differences between them are not at first obvious. Nevertheless, if the method of comparison is applied, I believe the result will show that VON MEYER's species is really a family including several species, and more than one genus.

The available data for comparison in the type specimen are remarkably scanty. It has been shown that the femur is 7.1 centims. long, and that it is seven times as long as the caudal vertebrae, which are almost uniformly 1 centim. long. The cervical vertebrae also yield some characters in the form of the neural arch and the ridges on the centrum.

There is no other specimen with the femur so short, but the differences in the length of the bone are so slight that they might at first pass for gradations of growth, their lengths in centims. being 7.1, 8.3, 8.8, 9.7, 10. All the bones, however, do not vary in the same ratio.

I will first contrast the type with the specimen described by LINK, known as the Waldenburg specimen. In that specimen (VON MEYER, *l.c.*, T. 9) the caudal vertebrae augment in length from 1.3 centim. in the early caudal to 1.8 centim. at the twenty-fourth caudal, where the specimen is fractured. The femur is 10 centims. long, but it

differs from the type in being as long as five-and-a-half of the middle caudal vertebræ, and as long as six-and-a-half of the early caudals. Moreover, the two femora are of different type; that in T. 9 being more expanded transversely at both the proximal and distal ends. The 23 caudal vertebræ of the type specimen measure as many centims.; the first 23 vertebræ in the tail in LINK's fossil measure 38 centims. If, on the evidence of the femur, the proportions of size between these animals may be taken as 7 to 10, then the 23 caudal vertebræ of the type would have measured 26·6 centims., or almost a sixth longer than is the case.

In the type I recognise two sacral vertebræ; in LINK's fossil there are three, according to VON MEYER. In the type I can only count sixteen vertebræ between the neck and the sacrum, where they measure 19 centims. In LINK's fossil there are not fewer than eighteen vertebræ in this region, measuring 30 centims. Seven-tenths of 30 being 21, it follows that the dorsal vertebræ in the type, besides being fewer, are relatively rather longer. The neural spines in the type are 6 millims. wide; here the width is double. The neural spines of the dorsal vertebræ in the type are 9 millims. high; here the height is about 2·2 centims. This is such a difference as might be attributed to age, but the aggregate of the other characters seems to me of specific value. I accordingly separate LINK's fossil as a distinct species, which may be termed *Protorosaurus Linkii*; until the discovery of better materials shall determine whether it can remain in the same genus.

Among other characters seen in LINK's specimen are all the details of the fore and hind limbs, showing the humerus to be 7·4 centims. long; the ulna 6·1 centims. long; the longest metacarpal about 1·9 centim. long, and the longest metatarsal 4·3 centims. The cervical vertebræ are relatively massive, 2·7 centims. long, and have the upper border of the neural arch nearly horizontal, without any trace of the posterior attenuation seen in the type of *Protorosaurus Speneri*. The caudal vertebræ show no transverse processes.

I would next compare SWEDENBORG'S specimen, figured by VON MEYER *l.c.* in T. 8. This is a smaller animal than LINK's, of less robust type. The cervical vertebræ resemble those of LINK's fossil in the contour of the neural arch, which is quite distinct from the College of Surgeons specimen, though there is more resemblance to the latter in the ridges on the centrum. VON MEYER's drawing, however, gives no indication of the possibly opisthocœlous articulation which appears to be indicated in the drawing of LINK's fossil. The shape of the femur is altogether Dinosaurian, and quite distinct from that in LINK's specimen, where it has a Crocodilian or Chelonian curvature. It is more than an eighth shorter than in LINK's type. The tibia is a sixth shorter. The metatarsus is a fourth shorter. The ulna is a fifth shorter. The cervical vertebræ are of the same length; the dorsal vertebræ one-eighth shorter; while the caudal vertebræ, which have transverse processes, have a uniform length of 1·1 centim., and are therefore relatively very short. The pelvic bones are badly preserved in SWEDENBORG'S fossil; but if the large expanded bones which lie in the sacral

region of LINK's fossil, beneath the humerus, are, as I believe from evidence in the British Museum, to be accounted pelvic, then the distinction between the types is very marked, and with the other characters would indicate a difference from LINK's specimen of more than specific value. SWEDENBORG's type is manifestly more nearly related to SPENER's type. If, as before, the comparison is based on the femur, SWEDENBORG's animal is larger than SPENER's in the proportion of 88 to 71. If, then, the dimensions in the latter are augmented by one-fourth, it should give approximately the size of the former. Four caudal vertebræ should measure about five centims.; they actually measure 5·5 centims. The correspondence is equally close in the proportions of the dorsal vertebræ. The chief differences in the cervical vertebræ are in form of the neural arches; but in length of centrum the correspondence, bone for bone, is exceedingly close between the theoretical measurements and the actual measurements. These resemblances seem to me to warrant the identification of the Vienna specimen with *Protorosaurus Speneri*. This determination makes known the hinder extremity of that species, the distal end of the humerus, the ulna and radius, the carpus, and some portion of the metacarpus. And it shows the complete series of dorsal ribs, to the distal ends of which slender sternal or abdominal ribs are articulated, two or three in number being placed side by side in connection with each dorsal rib. As the remains lie, they give a depth of body in this specimen of about 10 centims.

The specimens figured by VON MEYER *l.c.* on T. 6, with the exception of his copy from SPENER's figure, all belong to the same genus as *Protorosaurus Linkii*; but whether the species is identical, as would seem probable, I have not made the necessary calculations to determine. The Munich specimen, figured by VON MEYER *l.c.* in T. 1, fig. 1, though very fragmentary, is sufficiently different in some of its proportions to be worth comparison. The cervical vertebræ are preserved in sequence. They are of the same character as in *Protorosaurus Speneri*. Their lengths are given in the following Table, for comparison, in centims. :—

| | 1st. | 2nd. | 3rd. | 4th. | 5th. | 6th. | 7th. |
|-------------------------------|------|------|------|------|------|------|------|
| College of Surgeons | . | 1·9 | 1·9 | 2·5 | 2·4 | 2· | 1·8 |
| Vienna | . | 2·3 | 2·3 | 2·8 | 2·9 | 2·6 | 2·3 |
| Munich | ·6 | 2·0 | 2·6 | 3·1 | 2·8 | 2·8 | 2·3 |
| Freiberg | . | . | . | 2·5 | 2·5 | 2·3 | 2·2 |

From this it is evident that the vertebræ in the Munich fossil do not preserve a relation of proportionate length with those of *Protorosaurus Speneri*. Four dorsal vertebræ measure 5·9 centims., which is nearly the calculated length. The femur is 9·7 centims. long, has a rounded proximal end, and a slight sigmoid flexure, but is less massive at the ends and more slender than in *P. Linkii*, and not so straight or so wide at the proximal end as in *P. Speneri*. The humerus is imperfect, but the ulna

and radius are preserved. The width of the humerus corresponds very well with the SWEDENBORG specimen; but the ulna in the Munich specimen is 4·8 centims. long, while in the Vienna specimen it is 5 centims., so that the bone should have measured in this fossil about 5·5 centims. if the specimen had belonged to *Protorosaurus Speneri*. Hence this animal appears to differ from that species in being longer in the hind-legs and shorter in the fore-legs. But I do not venture to suggest for it a distinct name. It is interesting, as showing the coracoid and scapula.

Another specimen, preserved at Freiberg, is figured by VON MEYER l.c. in T. 2. It is of about the same size as the SPENER fossil, but only shows cervical and dorsal vertebræ, pelvis, scapular arch, and fore-limb. The cervical vertebræ are contrasted in measurement with the College of Surgeons specimen in the foregoing Table, from which it appears that the last two cervicals are relatively longer. Four dorsal centrums measure 4·5 centims., which would correspond with the length in SPENER's fossil. The bones of the fore-limb are slender and graceful in outline; and both ulna and radius are remarkable for a slight sigmoid flexure and constriction of the middle of the shaft. The humerus is 5·5 centims. long; the ulna and radius measure 4·6 centims., and the foot beyond the carpus about 5 centims. In the Vienna specimen the ulna and radius measure about 5 centims., and in the Munich specimen about 4·8 centims.; and in both are strong massive bones with sub-parallel sides and different contours. This character is suggestive of a specific difference; but, as the contours of the humerus are similar, and there is no fundamental difference in the form of the coracoid or of the pelvic bones, it seems to me more convenient to group the Freiberg fossil with *Protorosaurus Speneri* till it has been re-examined. It makes known the structure of a remarkable type of scapular arch, and gives some valuable details of pelvic structure.

The Berlin specimen, figured by VON MEYER l.c. in T. 4, has the femur 8·5 centims. long, while the tibia measures 9 centims. This reverses the usual relations of length between the leg and the fore-leg, and is probably a good specific character. The pelvic bones resemble those in the SWEDENBORG fossil, but differ in form. The femur is as long as six of the early caudal vertebræ. Further evidence is required to determine the systematic place of these remains. All the specimens hitherto compared are exposed in side view; but there are two other fossils figured by VON MEYER. One, preserved at Hanover, shows the dorsal aspect of the dorsal and sacral vertebræ, pelvis, &c.; the other exhibits the ventral aspect of the sacrum of a large animal preserved at Dresden. The Jugler fossil at Hanover has a femur of a massive oblong form, not unlike that in the Vienna fossil, but relatively much shorter and wider. It is about 5·3 centims. long and 2·2 centims. wide proximally, and is equal to the length of four dorsal vertebræ. In the Munich fossil the femur is equal to seven dorsal vertebræ, and the proportion is nearly the same in the Vienna specimen and in the College of Surgeons type. In the Waldenburg specimen, *P. Linkii*, the femur is as long as six dorsal vertebræ. But, although the relative shortness of the femur thus separates

the Jugler fossil from the others, the form of the femur is no less distinctive in its prop-like character and massive width. In *Protorosaurus Speneri* the bone is between four and five times as long as wide; here it is between two and three times as long as wide. The dorsal ribs shorten towards the sacrum in a way of which the Vienna fossil gives no indication, and which is not paralleled in any of the specimens referred to *Protorosaurus Speneri* or *P. Linkii*, since the last rib hardly exceeds the length of a dorsal vertebra. The ilium is in the form of an arch, the extremities of which rest against the bodies of the vertebræ, and to the middle of the outer curve of the arch the femur articulates. If all the vertebræ between the extremities of the arch are regarded as sacral, the sacrum includes five or six vertebræ at fewest. These characters are very scanty evidence of the animal, but they indicate in my judgment that the Jugler fossil belongs to a new genus and species. Till the genus is named the fossil may be referred to as *Protorosaurus Meyeri*. There can be no doubt that the Dresden fossil belongs to the same genus as the Jugler example, but I cannot at present determine whether it is specifically distinct.

From this discussion it appears that *Protorosaurus Speneri* as defined by VON MEYER included two or three genera and several species; and that the materials available for the elucidation of the type of the genus make known, more or less perfectly, the parts of the skeleton which are missing from the College of Surgeons specimen. The Jugler and Dresden specimens make known a form of pelvis as strong as anything met with among Ornithosaurs and fossil Reptiles, and show that the strength of the bones in the sacral region is associated with shortness and strength of the femur.

I now propose to use this evidence, brought together by VON MEYER in the discussion of the affinities and structure of the type of *Protorosaurus*.

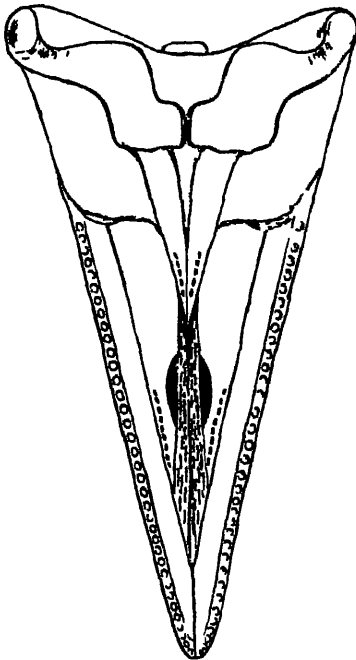
PART IV.

Comparison of Protorosaurus with other Types of Animals.

The skull.—Imperfect as is the preservation of the skull, it can be almost completely restored. The pterygoid bones, being still connected with the quadrate bones, furnish approximately the width of the palate in the transverse line of the quadrate articulation as not less than 4·5 centims., nor more than 6 centims. This is more than the width of the superior aspect of the back part of the skull as preserved, which would not have exceeded 2·5 centims., and if to this the width of the lost post-frontal and squamosal bones is added the width of the back of the skull presumably would still be less than the measurement over the condyles of the quadrate bones. The quadrate bones may have been inclined so as to converge upward, and thus have given an obliquely inclined aspect to the sides of the head, making its transverse section trapezoidal. If an attempt is made to reconstruct the palate (fig. 1), it is manifest that if the narrow internal facets of the pterygoid bones met each other

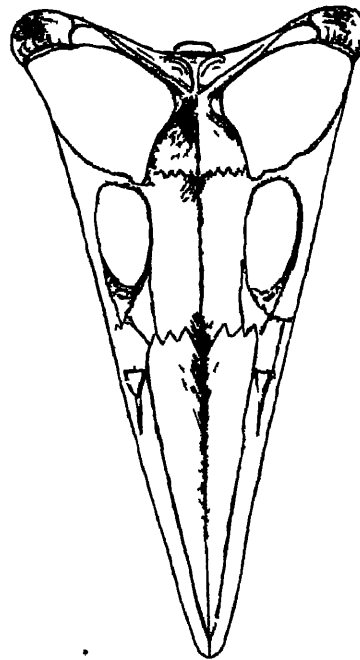
the bones diverged posteriorly. Hence I conclude that the sphenoid came between them after the type of *Ichthyosaurus*, and Lizards and Birds. The palatine bone shows some indication of having been slightly overlapped posteriorly as well as along one side of the anterior margin. The former character I take to indicate connection with the pterygoid bone, the latter with the vomer. We are entitled to infer on general analogy that the vomerine bones converged anteriorly. On this basis I reconstruct the palate with a slight median vacuity, less open than that of *Ichthyosaurus*; but there is no evidence whether there was a similar pre-sphenoid rostrum. There was a pair of lateral vacuities external to the pterygoid bones. The premaxillary bones and maxillary bones are necessarily external to the vomera and palatines, but their transverse width depends to some extent upon the

Fig 1.



Partial Restoration of the Palate of
Protorosaurus Speneri. Of the natural size.

Fig. 2.



Restoration of the Upper Surface of
the Skull of *Protorosaurus Speneri*.

degree to which the vomer laps along the palatine. In the restoration this parallelism of the two is represented, so as to carry the vomer far back; its position may have been more forward, and then the palatal plate of the premaxillary would have been narrower. The distance from the condyle of the quadrate bone to the extremity of the snout is 8.5 centims., that being the length from the articulation in the articular bone to the anterior extremity of the lower jaw, which is inferred to have extended as far forward as the snout. As the bones of the side of the jaw give no evidence of lateral constriction, the outline of the skull was an isosceles triangle or sugar-loaf contour.

The restoration of the upper surface of the skull is constructed on the basis of the

contour of the palate, upon which the measurements of the bones already described are drawn (fig. 2). The orbits of the eyes were possibly larger than here shown, while the posterior border to the orbit is given on the hypothesis that post-frontal and malar bones were present, and that the malar united with the maxillary in the usual way. There is no evidence whether the malar arch connected with the quadrate bone. The characters shown by the skull are not to be found in one order of animals. In the first case I will assume that the position of the nares is in the small ant-orbital vacuity, and that they were not terminal as in the South African Theriodonts described by Sir RICHARD OWEN, and in Chelonians, or subterminal as in existing Crocodilia and Ophidia. There are several fossil types in which the external nares are quite as small and have as backward a position. In *Pistosaurus* from the Muschelkalk both these conditions are seen, and in many species of *Plesiosaurus* the nares, and other vacuities of the superior surface of the skull, are similarly placed. In true Plesiosaurians there is not the same posterior constriction of the cerebral region, for the brain case is always widest in its hinder part. And Plesiosaurs have not the same broad flat interspace between the orbits formed by the frontal bones. But in *Nothosaurus* from the Muschelkalk there is the same posterior divergence of the occipital crest, a similarly inclined supra-occipital region, a corresponding posterior attenuation of the brain case, which, like the temporal vacuities, is more elongated; and there is a broad, flattened, inter-orbital area in *Nothosaurus*, though it is relatively smaller than in *Protorosaurus*, while the orbits are small, the nares relatively large, the snout not pointed, and there is a large parietal foramen. Altogether the resemblances are remarkable. The resemblances of the palate are less obvious, for no Plesiosaur or Nothosaur at present known has a palate which is open in the median line; though, so far as form is concerned, the pterygoid bones show some resemblance, and are noticeable for the width of the plate which laps along the quadrate bone in *Plesiosaurus*, though it is narrower than in *Protorosaurus* in proportion as the Plesiosaurian skull is more depressed.

The resemblance to the skull of *Ichthyosaurus*, in form, is very close. The orbits are behind the middle of the length of the head, and the temporal vacuities and nares are similarly situate; but there is a fundamental difference in the minute size of the true frontal bones in *Ichthyosaurus*, in which genus they are excluded from the orbital margin by the intervening nasal, post-frontal, and pre-frontal bones; moreover, the nasal bones do not usually extend in *Ichthyosaurus* nearly to the extremity of the snout. If, however, the Ichthyosaurian nasals had extended no further backward than in *Protorosaurus*, they would have come as far forward anteriorly, and if the frontal bones had grown to fill the space thus left vacant the post-frontal bones would have been pushed outward and backward; though there can be little probability that the post-orbital part of the Protorosaurian skull could have been Ichthyosaurian. On the palate the resemblance is greater than among Plesiosaurs, because the palate is open in the middle line in *Ichthyosaurus* and the bones are elongated and taper to their

extremities. The form of the pterygoid bone is quite as much like *Ichthyosaurus* as *Plesiosaurus*. But, though the superficial resemblance is more obvious with *Ichthyosaurus*, I believe the resemblances with *Plesiosaurus* are the more important.

A certain resemblance may be considered to be shown by the Triassic Crocodile *Belodon*; but that type, which has the orbits placed far back, has large ant-orbital vacuities, above which the external nares are situate; the nasal bones are exceedingly small and short; and the pterygoid bones which meet in the median line do not extend so far back as in *Protorosaurus*.

In none of the types with which comparison has been made are teeth ever present on the bones of the palate. The existing groups of Reptiles in which this character is seen are Ophidia, Lacertilia, and Rhynchocephalia, in all of which orders the external nares are terminal or sub-terminal. The same relation characterises the extinct Reptiles which have teeth on the bones of the palate, such as *Hyperodapedon* and *Rhynchosaurus*; and therefore, if as close a general resemblance should exist between such types and *Protorosaurus*, as the Nothosaurs have shown, the probabilities will incline to the anterior nares having been terminal. *Rhynchosaurus* apparently has teeth on both the palatine and pterygoid bones; the pterygoid is firmly united to the quadrate, the palate is open in the median line, but there is seemingly no very close resemblance to *Protorosaurus* in the forms of the bones. The upper surface of the skull is fairly comparable in contour, in the relative positions of the vacuities, in the broad, flat, frontal region, and in the existence of a parieto-frontal crest formed by the temporal muscles. The brain-case, however, in *Rhynchosaurus* appears to be distinct from the roof bones of the head, as in *Procolophon* and some Lizards; and this condition has no parallel in *Protorosaurus*. No existing Reptile, so far as I am aware, has teeth on the vomer; and this toothed condition of all the bones of the palate prevents detailed comparison being made with Lizards or Rhynchocephalia. The character, so common among Fishes and extinct Amphibia, is the more remarkable as a comparatively isolated resemblance to lower types. Besides its terminal pair of tusk-like incisors, *Rhynchosaurus* has apparently two parallel rows of teeth upon the palatal plate of the maxillary, and two short parallel longitudinal rows in the hinder part of the palate, which appear to be upon the pterygoid, or pterygoid and vomerine bones, for no separation can be made out with certainty between the bones of the palate. In *Rhynchosaurus* the malar bones are produced downward and backward so as partly to overlap the lower jaw, as in *Parieasaurus*, a character of which *Protorosaurus* gives no indication. *Rhynchosaurus* has the post-fronto-squamosal arch strongly developed, of which no trace is preserved in the *Protorosaurus*.

The condition of the teeth, ankylosed to the palate, with corresponding cavities in the positions where fangs would usually be, is a remarkable peculiarity, which needs further elucidation. The teeth are ankylosed to the jaw in *Labyrinthodonts*; but I know of no such union or such sub-dental loculi among Reptiles as are here seen. The cavities may have remained after the teeth emerged from them by absorption of

the base of the fang when the tooth became ankylosed. I am inclined to regard the attachment of the teeth as having more in common with Serpents and certain Lizards than any other group of existing animals; but the resemblance cannot be accounted one of affinity. And the question arises whether the sub-dental cavities are not to be interpreted as a stage in the history of the formation of the socket for alveolar teeth, which became developed in a later period of time. If there had been sufficient evidence to establish this interpretation, it would have tended to make *Protorosaurus* comparable with some Ornithosaurs from Solenhofen, to which resemblances may be seen in the general structure of the skull and conformation of the palatal bones, though no Ornithosaur has teeth on the bones of the palate.

The vertebral column.—The atlas is very short and not ankylosed to the axis. There are about 7 cervical vertebræ: 16 dorsal; two or three sacral; and an unknown, but large, number in the tail. These numbers throw no light on the affinities of the fossil. The elongation of the cervical vertebræ, as VON MEYER pointed out, is better paralleled among Ornithosaurs than any other group. The strongly developed neural spine is not found in all members of this group, but is sufficiently characteristic. VON MEYER's figures appear to show that the vertebræ have the articular ends of the centrum slightly concave; and therefore it is probable that if the opisthocœlous condition which the type appears to show is not delusive the form of the articulation is not constant. I do not know of any Ornithosaur which has a like antero-posterior elongation of the neural spine. The cervical ribs are Ornithosaurian. The dorsal vertebræ are remarkable for the rounded base and depth of the centrum, which supports a large neural arch with vertical truncated neural spine. This form of vertebra approximates rather to the Crocodilian than the Lacertilian type, but is

Fig. 3.

Fourth cervical vertebra of *Protorosaurus Spaneri*.

better matched among existing Birds with amphicœlous vertebræ, Pterodactyles, Dinosaurs, and Nothosaurs, though it never has anything like a neural platform; and there is no certain evidence of any ribs having more than one articular head, though the articulation of the rib was quite as high in lateral position as among Crocodiles. On the whole, the Ornithosaur comes closest, though *Rhynchosaurus*, in the form of the centrum, is not dissimilar. There is no approximation to the massive neural arch of *Nothosaurus*, and the centrum is more elongated than in that genus.

The sacrum presents no peculiarities; and, although only two pairs of strong sacral ribs were developed to support the ilium, that bone appears to have been sufficiently

extended to have articulated with more. The tail is equally devoid of characters which suggest affinities. In depth the centrum has more in common with the Crocodilian and Dinosaurian types than with Nothosaurs, which have the centrum shorter, or with Lacertilians or Ornithosaurs, which have it more depressed. The divided condition seen in the neural spine of the later caudal vertebræ probably indicates a complete development of the neural arch upon each of the protovertebral elements which go to make up the centrum; though the vertebra must still be regarded as highly differentiated, since the caudal ribs are given off from its anterior moiety as processes directed transversely, while the chevron bones, which represent them on the posterior moiety, have already descended to the inferior visceral margin. The theory of the double-headed articulation of dorsal and cervical ribs is not unconnected in some animals with the hypothesis that the transverse process and chevron bone blend in the anterior part of the body to form one rib with two articulations, and sometimes with a pair of sternal ribs to each distal extremity. The attachment of the ribs being high up, as well as the length of the ribs, would indicate that the respiratory and vital organs in *Protorosaurus* were well developed; and the mode of attachment of the ribs in most Reptiles and higher Vertebrates appears to depend partly on the way in which they are elevated by the lungs, and partly on the muscles which come into play in connecting the ribs with the vertebræ. So that the double-headed attachment of the dorsal ribs in modern Crocodiles is fundamentally different from the attachment in Birds, only because the transverse processes have become so much elongated as to remove the rib from the side of the centrum. But the Mammalian and Avian ribs are typically single-headed, and the second head or tubercle is obviously only a consequence of the rib being brought into contact with the neural arch; so that, if no transverse platform is developed, the rib cannot have a second articulation. And it is on this condition that I account for the single-headed ribs of *Protorosaurus*, since nothing is needed to make the rib double-headed but a transverse development of the neural arch, such as I shall subsequently describe as partially developed in the genus *Mesosaurus*.

The sternal ribs are imperfectly known. VON MEYER represents them as rods, of which two, or possibly three in some cases, are attached to the enlarged sternal end of each costal rib. The sternal ribs, however, were probably composite; and I am disposed to believe that each consisted of two lateral pieces on each side, united by squamous overlap with a median piece in the middle line of the abdomen. Sternal ribs are seen in *Lariosaurus*, in *Mesosaurus*, in *Stereosternum*, in *Rhynchosaurus*, and other Triassic and Permian types, as well as in *Plesiosaurus*. Their structure is in every case substantially the same when it can be observed; though the number of sternal ribs to each costal rib varies. The nearest approximation to this condition among existing Reptiles is seen in *Hatteria*. I have no doubt *Rhynchosaurus* is a Rhynchocephalian; but *Lariosaurus*, *Mesosaurus*, &c., are Nothosaurians.

The pelvis and hind-limb.—The pelvis is not complete in any specimen. But the ilium appears from the sum of the evidence to have an antero-posterior elongation

and a distinctive form, but was more nearly comparable with the bone in Solenhofen Pterodactyles than in any other kind of animal, living or extinct.*

It is probable that the bone was supported as an arch, of which the extremities met the bodies of vertebræ, and the middle was attached to sacral ribs. In this matter we are not entitled to reason from the Hanover and Dresden specimens, because they have already been regarded as referable to another, though allied, genus; but the ilium is always broken and more or less displaced, and this favours the view that it was arched as in those types. The acetabulum is unknown. The ischium and pubis are altogether Pterodactylian, being expanded bones essentially comparable in contours and mode of union, with an obturator foramen between them; the pubis smaller than the ischium, with the ilium extending anteriorly and posteriorly beyond both bones. Something of the same type of pelvis is seen among Anomodonts, and

Fig. 4.



Nothosaurians and Plesiosaurians show a similarity in the ischium and pubis. It is also paralleled among the Cetiosaurian Dinosaurs, and after the Ornithosaurs those Dinosaurs would approximate most closely to *Protorosaurus* in pelvic structure. This pelvis is more comparable to the Crocodilian than to the Lacertilian type. So far as the ilium is concerned, an approach to this type is made by the Jurassic *Scaphosaurus*.

The femur, as already remarked, is Dinosaurian in its straight strong build, truncated proximal end, and distal condyles. If it has not the lateral trochanter or the proximal trochanter usual in Dinosaurs, the latter is absent from the femur of so typical a Dinosaur as *Hadrosaurus*, and the former is absent in *Stegosaurus* and some other American genera.

An approximation to this form of femur is found among Nothosaurians, but those animals never exhibit the perfect ossification of the extremities here seen, or distal

* SEELEY, 'The Ornithosauria,' p. 60, 1870.

condyles. The second segment of the limb is more like that of a Dinosaur than any other animal. Very few Dinosaurs have the tibia and fibula as long as the femur, but Professor MARSH has figured this condition in *Laosaurus*; and in *Compsognathus* the relative elongation of the second segment of the limb is greater than in *Protorosaurus*. Ornithosaurs also have this segment of the limb the longer, but then the fibula is only developed proximally, as among Birds. The proximal expansion of the tibia in *Protorosaurus* and its cnemial crest, well seen in the type specimen, are typically Dinosaurian (fig. 4). The sigmoid flexure seen in the fibula in some examples of *Protorosaurus* suggests that the bone terminated distally in front of the tibia, as in *Archæopteryx* and certain Dinosaurs.

The tarsus differs from that seen in Dinosaurs in some important particulars. First there is a large astragalus which appears in the SWEDENBORG specimen to have an ascending talon; then there is a compressed calcaneum, which in the Waldenburg specimen articulates with the cuboid bone. Between this proximal row and the distal row of three cuneiform bones are the cuboid and naviculare. This remarkably well developed tarsus is distinctive of *Protorosaurus*, and Mammalian in its elements.

There are five stout metatarsals, which increase in length from the first to the fourth, while the fifth is but little longer than the first. They do not decrease in stoutness, as is the case with Crocodiles. They are more elongated than among Lizards, though *Scaphæosaurus** has similar bones, but shorter than in *Compsognathus* and some Ornithosaurs, like *Dimorphodon*. No near parallelism is possible with Dinosaurs, because the bones of the digits are so greatly elongated in *Protorosaurus*, for this condition gives a Lacertilian character to the hind-limb, though the stoutness of the phalanges and metatarsals is more Dinosaurian. From the shortness of the first and fifth digits, and especially of the metatarsals of those digits, the foot has a suggestive aspect of degeneration, which, when the metatarsals came to be carried in an elevated position, might result in the development of such a foot as is seen in *Allosaurus*, though the fact that the fourth metatarsal is the longest seems to offer some difficulty in the simplification of the Protorosaurian foot. The digital bones are ossified on the Dinosaurian type.

The shoulder-girdle and fore-limb.—The shoulder-girdle is less perfectly preserved than the pelvis. Bones which I regard as the coracoid and scapula are preserved in the Munich specimen. If these are traced off and articulated, they show that the coracoid was relatively large, but, like the scapula, is suggestive of Dinosaurian or Ichthyosaurian form, though the coracoid is not without some resemblance to the bone in Plesiosaurs, and Plesiosaurs always want the narrow anterior notch. Only a trace of these bones is seen in the Vienna specimen, where the very imperfect coracoid and scapula appear to have had similar forms; but there is no evidence whether the coracoids met in the median line, or whether other bones

* VON MEYER, 'Fauna der Vorwelt,' "Reptilien aus dem Lithographischen Schiefer des Jura," t. xiii.

extended between them. Much more complete remains of the shoulder-girdle are preserved in the Freiberg specimen, but the bones figured by VON MEYER are not easily understood, and are very different from the bones in the Munich fossil. They are probably displaced, and till I have examined the original can offer no decisive opinion on structures which appear to unite the characters of Plesiosaurs and

Fig. 5.



Dinosaurs with distinctive ordinal characters. The scapula is formed on the Nothosaurian type, while the coracoid is unlike that of any Nothosaur, and might be Lacertilian or Dinosaurian. And the arch appears to include other elements, which are probably the interclavicle and clavicles; so that the resemblance to Dinosaurs which appears to be indicated by the bones in the Munich slab may have to be modified in favour of a more generalised interpretation of affinity.

The fore-limb is much smaller than the hind-limb. The form of the humerus with its expanded ends might be Rhynchocephalian, Lacertilian, or Dinosaurian. One large humerus figured by VON MEYER as the Fulda specimen is quite Dinosaurian and has a large radial crest, but there is no proof that this can be referred to the same genus as SPENER's fossil, though the small specimens appear to have a similar form and to possess a radial crest; but this might be Lacertilian as well as Dinosaurian. The smaller limb bones are equally remarkable for wanting characters suggestive of definite affinity with existing Reptiles. They are not Crocodilian, not like any Lizard known to me, and not typically Dinosaurian, but only to be described as of generalised type. The carpus consists of rounded bones, of which five form a distal row corresponding to the five metatarsals, and three the proximal row, which lies on the radial side, so that the ulna appears to articulate directly with the fifth distal carpal. If in form of the bones the carpus appears Plesiosaurian, it is as much Cetacean in that respect, and in structure makes as near an approach to Mammalian type as to Reptiles.

The metacarpus has the bones more nearly equal in length than the metatarsus; three are fasciculated in the middle, and the inner and outer bones are shorter and more spread out laterally.

From this discussion I conclude that *Protorosaurus* has no predominant affinity with any existing order of animals. Its cranial characters appear to separate it widely from other ordinal groups. If the strongest resemblance of the upper surface of the skull is with certain Nothosaurs, the dental characters separate it. The second strongest resemblance is probably with certain Jurassic Ornithosaurs. The vertebral column as a whole has much in common with Pterodactyles, more perhaps than with any other group, but the differences in the articulation of the ribs and the sacrum separate it. The pelvis is intermediate between that of Pterodactyles and Plesiosaurs or Nothosaurs. The hind-limb is in its proximal segments suggestive of Dinosaurs, and in its distal segments approximates to Lizards. The scapular arch is too imperfectly known to yield marked evidence of affinity. The fore-limb shows no striking differentiation. The animal is therefore of an ancient stock, and may have been derived from the group from which Ornithosaurs were developed. Hence I conclude that VON MEYER was fully justified in regarding *Protorosaurus* as the type of a distinct order of Reptiles, for which the name Protorosauria may be conveniently used.

DESCRIPTION OF THE PLATES.

PLATE 14.

Figure of specimen in College of Surgeons (No. 308, natural size).

Figs. 2-7. Cervical vertebræ: *sa*, transverse process of sacral vertebra; *f*, femur; *fb*, fibula; *t*, tibia; *a*, dermal armour.

PLATE 15.

Enlargement of skull and details of the teeth.

Fig. 1. *n*, nasal; *f*, frontal; *p*, parietal; *so*, supra-occipital; *pf*, prefrontal; *pm*, premaxillary; *s*, ? sclerotic armature; *q*, quadrate bone; *pt*, pterygoid bone; *pl*, palatine bone; *v*, vomer; *d*, dentary bone; *a*, articular bone; *an*, angular; *san*, surangular; *sp*, spleniate.

Figs. 2, 3. Teeth in skull.

Figs. 4, 5. Teeth on the pterygoid bone.

Figs. 6, 7. Teeth on the vomerine bones.

Figs. 8, 9, 10. Teeth in the lower jaw. The appearance of a fang in fig. 9 may result from the alveolar border rising above the base of the tooth.

Fig. 11. Dermal armour.

PLATE 16.

Outline restoration of the skeleton of *Protorosaurus* reduced. The shoulder girdle is omitted. The restoration is based chiefly upon the Vienna specimen.

X. On the Action of the Excised Mammalian Heart.

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§ I. *Introduction.*

Our attention was directed to the action of the excised Mammalian heart in the course of experiments relating to the electromotive action of various tissues, by our observation of the fact that the electromotive variations indicative of action outlast visible contractions; secondly, that the contractions of the excised organ are of an extraordinarily prolonged character.

We consequently pursued our observations (1) by the galvanometer, (2) by the aid of the graphic method, (3) by the capillary electrometer (LIPP MANN). By the galvanometer and by the electrometer we observed the electrical changes consequent upon spontaneous or provoked contraction, and in the absence of visible contraction. By the graphic method we determined the duration of spontaneous and of excited beats, and, with regard to the latter, the length of the latent period at various times after excision. Incidentally we also observed the time during which the rhythmic beat persisted after excision and the character of that beat under varying conditions of temperature. By both methods we were able in favourable cases to follow the progress of the wave of contraction, both spontaneous and excited. Our observations were made on the hearts of Cats, Dogs, Rabbits, Guinea-pigs, Rats, and Sheep.

§ II. *The during which spontaneous contractions continue after excision of the heart.—Mode of decline.*

The statements as regards this point which are most generally quoted are based upon the observations of CZERMAK and PIOTROWSKY, who found for the heart's beat of

Rabbits a minimum persistence of 3 minutes, a maximum persistence of 36 minutes; the mean of 60 observations was 11 minutes 46 seconds.* In the few observations which we devoted to this point we obtained durations considerably longer than the maximum given above.

We obtained, for instance, from three Rabbits' hearts the record of spontaneous beats during 72 minutes, 71 minutes, and 42 minutes respectively, and beats in response to excitation for as long a time as $1\frac{1}{2}$ hours post mortem (Nov. 28). On Cats we recorded spontaneous beats for periods of $25\frac{1}{2}$ minutes (Nov. 30), 23 minutes (Dec. 2). On a Dog we observed them for 2 hours after excision. These periods were longer than we anticipated, but they were exceeded by a figure given by ROUSSEAU in 1808, viz., 29 hours post mortem,—contractions on a guillotined Woman,† also by figures given by VULPIAN, $93\frac{1}{2}$ hours (R. Auricle of Dog), $46\frac{1}{2}$ hours (Auricles of Rat).‡ BROWN-SÉQUARD also gives some very high figures, 53 hours (Dog), 34 hours (Rabbit), 31 hours (Guinea-pig).‡

It must be observed, however, that in most cases the heart remained *in situ*, that generally the movements observed were of the R. Aur. or of the Vena Cava, and that, as far as can be gathered from the description, the observations were generally of the fibrillation of minute portions placed under the microscope. Our observations, except in the case of the Dog's heart above mentioned, were of complete ventricular contractions giving characteristic records. The heart was removed immediately after decapitation and placed upon an adjustable platform with a light lever resting upon it, and our results are comparable with those of CZERMAK and PIOTROWSKY,* with this addition that we recorded the movements.

Of fibrillation such as that spoken of by the French physiologists we took no account; we noticed it indeed, but did not follow it to its end.

We are not acquainted with any such published records of the movements of the excised Mammalian heart, we therefore submit some examples illustrative of the usual mode of the decline and of its ordinary modifications at various times post mortem. (*Vide* Tracings 1, 2, 3, 4. Tables A, B.)

1. Generally speaking, the decline is fairly regular as to force and frequency of contraction; force of contraction declines, however, more rapidly in the first moments after excision than at later periods (Tracing 1); frequency of contraction diminishes throughout the observation regularly at first (Tables A and B); at a later period the contractions are at long and irregular intervals (Table D).

2. The supervention of a bigeminal character is frequent as a regular irregularity. Sometimes the beat is bigeminal from the instant of excision to the end of observation, sometimes it is uniform at first and gradually becomes bigeminal. The bigeminal

* 'Wien, Akad. Sitzber.,' vol. 25, 1857, p. 431.

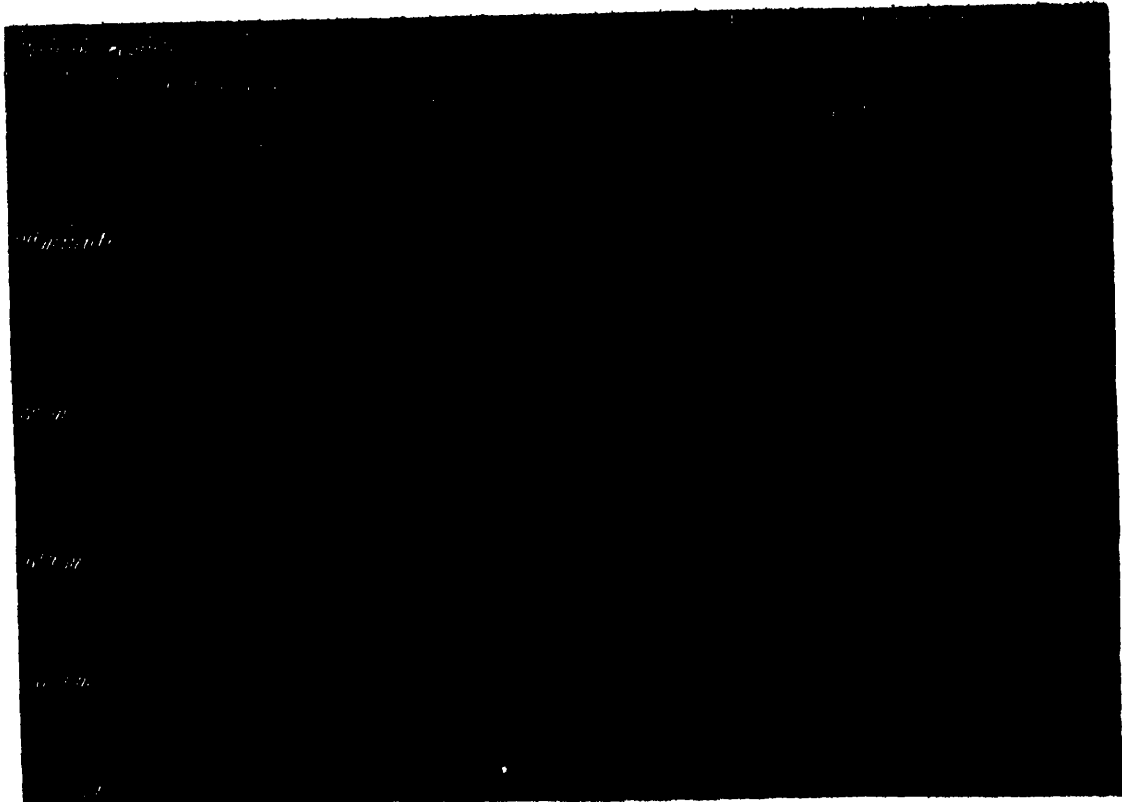
† 'Comptes Rendus,' 1858, p. 3.

‡ 'Journal de la Physiologie,' vol. 1, 1858, p. 357.

character is more pronounced at the base than at the apex, and may be entirely absent from the auricle when it is present in the ventricle. (Tracings 1, 2, 3.)

3. A third feature is sometimes very marked in such cases where the decline in force and frequency is not regular. A pause of unusual length is followed by a beat

Tracing 1.



Cat's heart. Record begins 1 minute, ends 18 minutes, post mortem. Diastolic supervision at end of 1st minute, and continued so until 18th minute, when delirium cordis supervened. Reduced.

NOTE.—The tracings on smoked paper were taken on a cylinder having three rates of revolution. All tracings read from left to right.

1. Quick rate, 1 mm. of surface = .004 second.
2. Middle rate, 1 mm. of surface = .022 second.
3. Slow rate, 1 mm. of surface = .15 second.

Tracings 1 to 8 inclusive are taken with the slow rate. Tracings 9, 10, 11, 14, and 15 inclusive are taken with the middle rate. Tracings 12 and 13 inclusive are taken with the quick rate.

of unusual height (Table C, Tracing 4). This sign of restorative action during rest occurred with very different frequencies. It is noteworthy, however, that in bigeminal tracings stronger contraction is not preceded by a longer pause, nor the weaker contraction by a shorter pause.

4. Beats in groups are sometimes seen as an irregularity (Tracing 4).

5. Extreme irregularity in the form of irregular rapid fibrillation (delirium cordis) occasionally occurred (Tracing 1).

6. In the first few minutes after excision the sequence of auriculo-ventricular

Tracing 2.



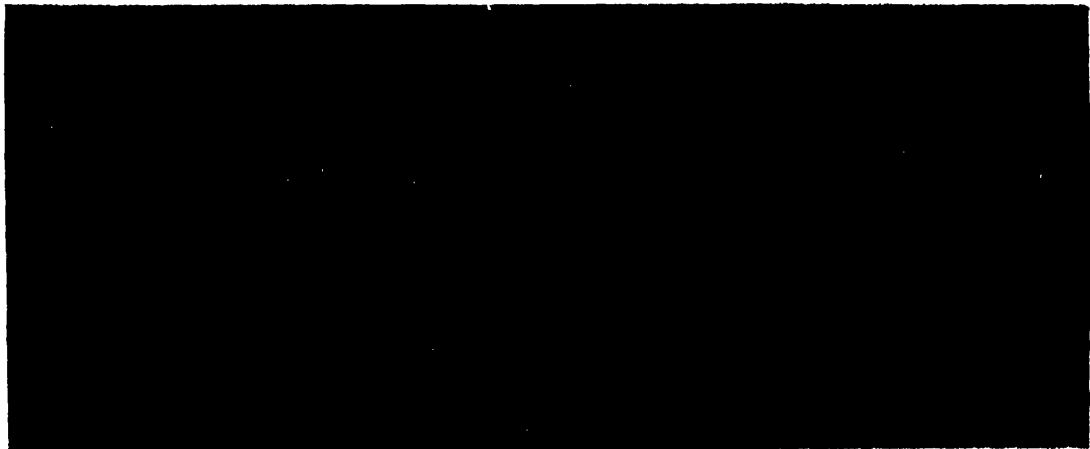
Tracing of Cat's hearts, 5 minutes post mortem, showing bigeminal character of beat, taken by a double cardiograph consisting of two levers resting upon the ventricle near the base and near the apex; the bigeminal character is more marked near the base than near the apex.

Tracing 3.



Rabbit's heart; double cardiograph, consisting of two levers resting on auricle and ventricle; the bigeminal character is evident in the ventricle, while it is absent in the auricle. (We have never seen it in the auricle.)

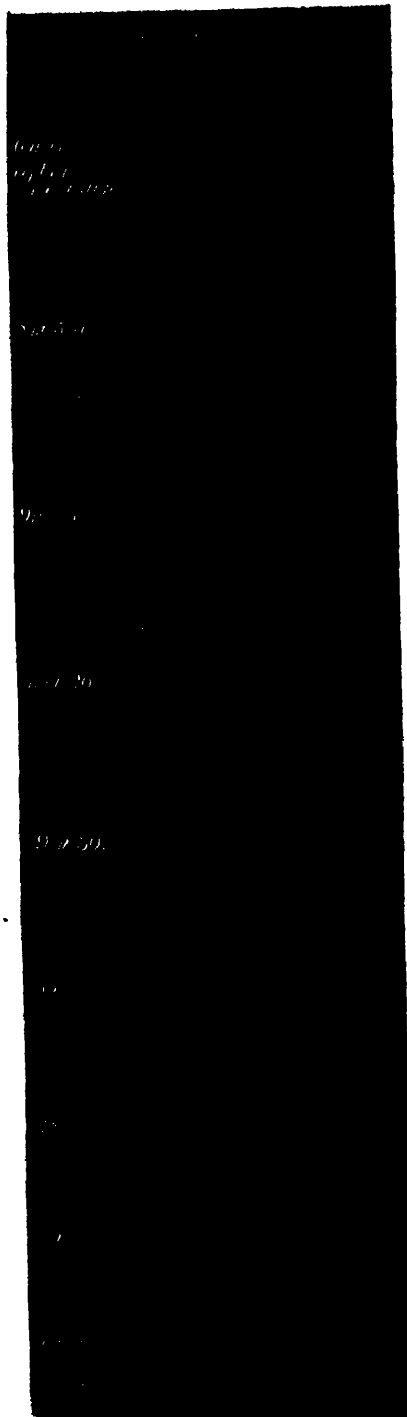
Tracing 4.



Cat's ventricle; record commences 3 minutes post mortem; shows ventricular contractions in groups separated by pauses, each pause being followed by a contraction of unusual strength.

action was normal. Later several auricular beats occurred to one ventricular beat (Table D). Later still the ventricular beat was quite independent of the auricular beat. We incidentally observed the ventricular action to outlast the auricular.

Tracing 5.



Contractions of the excised Rabbit's heart from the 6th to the 75th minute after excision; showing increasing prolongation of the ventricular contraction.

The two points to which we specially directed our attention at this stage were the changes in the duration of the ventricular contraction, both spontaneous and excited, and in the length of the latent period of stimulation. With regard to previous observations, we know of none, with the exception of a very imperfect datum consigned in LANDOIS' "Physiologie," 1880, p. 95, where he gives the maximum duration of the systole of the excised Rabbit's heart as .48 second, the normal duration being .25. Of the period of latent stimulation we have nowhere found any mention.*

§ III. *Duration of systole in the excised heart.*

Our observations show that from the moment of excision the ventricular contractions steadily increase in duration to a maximum of about 6 seconds, the normal duration being about .3 second. This statement holds good for hearts of Cats, Dogs, and Rabbits removed and observed at ordinary room-temperature (16° Cent.). The hearts of these animals at 16° Cent. give contractions far exceeding in duration that of the contraction of the Frog's or Tortoise's heart at the same temperature. Table A and Tracing 5 are illustrative of this statement.

The chief cause of this change is an alteration of temperature, the heart *in situ* being at a temperature of 37° to 40°, while the temperature of the room was between 15° and 18°, or during the winter as low as 12°. That the temperature factor was

* Three times in the course of our observations we noticed that immediately after decapitation the diaphragm on the left side contracted simultaneously with the beat of heart. In one case the contractions of the diaphragm were so strong as to bend the body of the animal to the left side with each beat of the heart. We supposed that the phenomenon was one of secondary contraction, the left phrenic being excited by the electromotive change of the heart at each contraction. The contractions of the diaphragm were at once arrested by section of the phrenic below the heart.

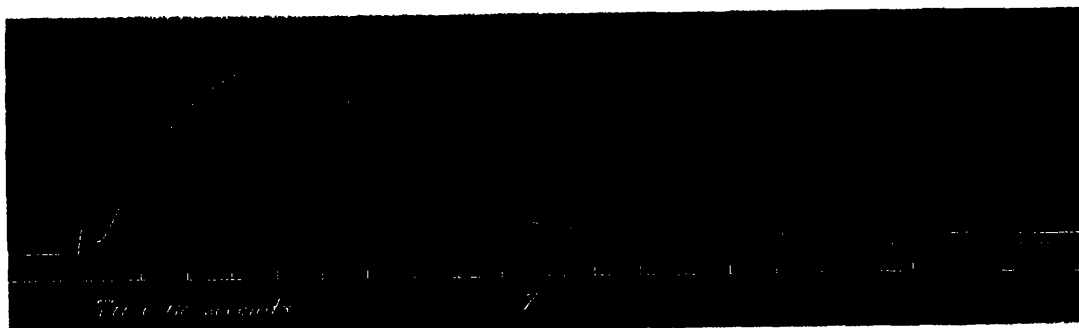
the important one was shown, 1st, by placing the heart in a constant temperature chamber at 40° , 2nd, by placing it in a chamber surrounded by ice. In the first case the characteristic prolongation was almost entirely absent, in the second it was greatly exaggerated. (Tracing 6.)

Tracing 6.



Beats of Dog's heart in warm chamber at 38° C.; the decline is rapid and there is no appreciable prolongation of the ventricular contraction.

Tracing 7.



Effect of cold (8° C.) on ventricular contraction of a Kitten's heart; the latent period is about .5 second, the duration of contraction is at least 10 seconds (this is the greatest prolongation that we have observed).

We do not think, however, that the temperature is the only condition involved, though it is certainly the chief one, for we have, as a rare exception, observed an alternately longer and shorter contraction, without of course any possible alternation of temperature, and we have found that the hearts of very young animals are more susceptible of this modification than those of fully grown animals. (Tracing 7.) An example of the excessive prolongation of which the contraction is capable under the influence of low temperature is furnished by Tracing 8.

In the course of our experiments regarding the effect of temperature upon the heart's contraction, we made observations showing the great susceptibility of the Mammalian heart to cold, and the preservative influence of cold upon its capacity for action. The effects of cold (surrounding by melting ice) are, in the order in which

they occur, 1st, lengthening of the spontaneous contractions; 2nd, abolition of the spontaneous contractions; 3rd, diminished excitability to mechanical* stimuli, with lengthening in the period of latent stimulation; 4th, abolition of excitability to all stimuli. The full effect of cold having been produced, viz., total abolition of contractility, spontaneous and provoked, the application of warmth (by surrounding the vessel containing the heart with water at 40° C.) can restore contractility, spontaneous as well as provoked, the latter reappearing before the former. This abolition of contractility by cold, and its restoration by warmth, may be repeated more than once; we have repeated it as many as three times. The following experiments illustrate these statements:—

Experiment I.—Kitten's heart. December 5th, 1885. Record began $1\frac{1}{2}$ minute after decapitation. Heart in small crucible surrounded by ice. The heart at first gave 11 beats during the first 7 seconds; it subsequently gave strong contractions at very long intervals (about 20 seconds) for a period of $4\frac{1}{2}$ minutes.

It continued excitable for 6 minutes longer. During the last minute of this period the prolongation of the latent period and of the contraction were excessive, greater than any we had hitherto observed; the latent period was about 2 seconds, and the length of contraction was so great and its fall so gradual that it could not be exactly measured; it is reproduced in Tracing 8. Its rise to a maximum occupies nearly 4 seconds. At end of 12 minutes post mortem, it was no longer excitable.

The ice was removed and replaced by warm water at 40° C. The heart became excitable, but spontaneous beats did not return.

The latent period was so short that we did not measure it.

The duration of contraction was shortened, and it was noteworthy that the ascent of the curve was rapid while its descent was still gradual.

Experiment II.—Rabbit's heart. December 5th. The record began 1 minute after decapitation, the heart being in a cup surrounded by ice.

It only beat for $1\frac{1}{2}$ minute, giving altogether 13 spontaneous beats.

It continued excitable to direct stimulation for about 3 minutes longer.

After the heart had ceased to be excitable it was removed from the influence of the ice, and was subjected to that of warm water at 40° C.

Within 1 minute of application of warmth the heart recommenced to beat *spontaneously*—at first slowly, afterwards more rapidly.

Excited contractions followed so rapidly upon mechanical excitation that we could not measure the latent period with the rate of revolution employed.

| Minutes after application of warmth. | Frequency of beat. |
|--------------------------------------|-------------------------|
| 3 min. | 8 beats per 15 seconds. |
| 5 " | 6 " " " " |
| 8 " | 3 " " " " |
| 11 " | 2 " " " " |
| 14 " | 2 " " " " |

Observation stops 30 minutes post mortem, the heart still beating feebly.

It is noteworthy that after the application of cold during 10 minutes, by which spontaneous contrac-

* We found mechanical stimulation more effectual as well as more convenient than stimuli from the induction coil.

tions and excitability were entirely abolished, the heart, under influence of moderate warmth, recovered so as to beat for 20 minutes, giving during that period more than 150 beats.

Experiment III.—Small Rabbit. December 18th, 1885. Decapitated at 12. Heart excised and placed in porcelain crucible surrounded by ice.

| Time. | |
|-----------|---|
| 12.2. | Record begins. |
| 12.7. | Spontaneous beats have ceased. |
| 12.9. | Excitability has ceased. |
| 12.13. | Warmth applied (water at 40°). |
| 12.14. | Spontaneous beats renewed. |
| 12.20. | Cold applied (melting ice). |
| 12.24. | Spontaneous beats ceased. |
| 12.26. | Excitability ceased. |
| 12.28. | Warmth applied (40°). |
| 12.28-30. | Spontaneous beats. |
| 12.40. | Cold applied (0°). |
| 12.44. | Excitability ceased. |
| 12.48. | Warmth (40°). |
| 12.50. | Spontaneous beats. |
| 12.55. | Excitation, followed by "delirium." |
| 1 | Cold applied for 10 minutes; quite inexcitable. |
| 1.10. | Warmth applied; a single spontaneous beat. |
| 1.20. | Heart quiescent and inexcitable. |

Remark.—The excitability of the heart was three times in succession abolished by cold and restored by warmth.

We have gone further than this: we have frozen the heart until it was quite hard by placing it in a capsule surrounded by ice and salt, and have observed its spontaneous contractions when it has been thawed.

Experiment IV.—December 19th, 1885. A Cat was decapitated at 11 59, the heart quickly excised, and placed in a porcelain capsule surrounded by ice and salt; by 12.14 it was frozen hard; it was removed from the freezing mixture and placed in another capsule surrounded by water at 42° C. It commenced to beat spontaneously at 12.24½, and a lever was at once placed upon it, and a record taken on the smoked cylinder. 20 spontaneous beats were recorded, and the heart remained excitable to the prick of a needle till 12.33.

We have gone further still: we have left the freshly excised heart in a freezing mixture for 3 hours. Its contractility did not, however, return. The heart's beat was recorded on the smoked cylinder in all the above experiments.

Warmth, notwithstanding its restorative effect upon a cooled heart, is not favourable to the long persistence of its rhythmic contraction after excision, nor even if it be left in the body of the recently killed animal. We have noticed at the outset that the excised and cooling heart usually beats for a longer period than a heart left in the body; we observed later that an excised heart beats for only a very short period in the warm chamber at 38° C., and we may add that a small, and therefore rapidly cooled, heart (Rabbit) outlasts a large and slowly cooling heart (Sheep). As of other tissues,

the excitability of the heart is great, and its decline rapid, at high temperature, while excitability is small, and decline slow, at low temperature.

§ IV. *The latent period of stimulation.*

Our observations show that there is a general correspondence between the duration of contraction of the ventricle, and its excitability, and the length of the latent period of stimulation.

In general, the correspondence is such that, with the lengthening of the contraction, excitability decreases, and *vice versa*. That these effects are in the main dependent on temperature is shown by the fact that they can be altered at will by variations of the temperature of the surrounding medium. Thus we may alternately obtain with the same heart, 1st, long contraction, long latent period, and obtuse excitability, or 2nd, short contraction, short latent period, and acute excitability, at (1st) lowered or (2nd) heightened temperature respectively.

A heart removed from the body, and examined at 15° to 18° C., shows the gradually increasing changes characteristic of lowered temperature; independently of this factor, however, quite similar changes supervene which are to be referred to the natural decline of action from the life normal to the death zero. This decline is, as for other tissues, not instantaneous, but gradual, and characterised by gradually increasing sluggishness, first, of spontaneous action, and secondly, of responsive action. The complete elimination of the temperature factor did not enter into the plan of our observations, and we give no experiments in support of the above general proposition, although we have observations sufficient to justify our assertion of it as of an experienced fact, not merely of an untested truism. The fact which we desired to clearly demonstrate is the great sluggishness of action to which the Mammalian heart may be reduced before action is extinguished; so that its processes, normally far more rapid than those of the cold-blooded heart, become so protracted as not only to equal, but to exceed, the latter in slowness of accomplishment. Our observations furnished the demonstration, and clearly show that the Mammalian heart is more susceptible to differences of temperature than is that of the Frog.*

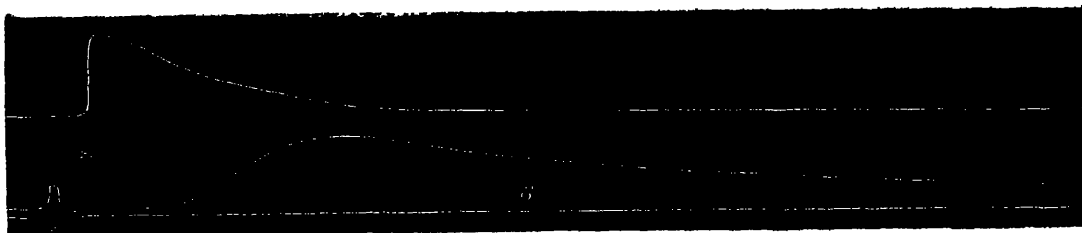
Of the statements regarding the three features referred to above, viz., length of systole, length of latent period, degree of excitability, the first has been considered in § 1; with regard to the third, we have no evidence to give beyond the statement that

| | | |
|--------------------------------------|---------|--|
| | 27°—0·9 | } BURDON SANDERSON, 'Journal of Physiology,' vol. 2, p. 384. |
| | 24°—1 | |
| | 21°—1·3 | |
| | 18°—1·6 | |
| Normal duration of Frog's systole at | 15°—1·9 | |
| | 12°—2·1 | |

Frog's systole ten times as long at 3° to 7° as at 18° to 20°. HERMANN, vol. 4, p. 372.

we could roughly estimate excitability by the greater or less energy with which a mechanical stimulus is required to be applied to provoke contraction; with what we have termed "acute" excitability, the lightest superficial touch was sufficient to discharge a beat; with "obtuse" excitability, pricking or scratching with a needle was required to produce any effect; between these two extremes we could and did test gradations which justified our statement. To the second feature, viz., length of latent period, we paid particular attention; a summary of our results is given in Table E, from which it appears that the latent period is prolonged with prolongation of the heart's contraction. There is a rough relationship between the two magnitudes, the former being to the latter about one-tenth; but the relationship is only a rough one, owing to the fact that when the contraction is very prolonged it is impossible to determine where it ends on the tracing, and its true time-value cannot, therefore, be exactly given. But the chief point is unmistakably clear: the latent period of stimulation may be extraordinarily prolonged, a very usual length being about .5 sec., and an exceptional maximum being as great as 2 secs. (see Tracing 8). This, as far as

Tracing 8.



Rabbit's heart; effects of cold and heat on latent period; in the cooled heart the latent period is about 2 seconds, the ventricular contraction rises slowly to its maximum; in the case of the warmed heart the latent period is very short, and the ventricular contraction very rapidly reaches its maximum; the decline is in both cases gradual, most so in the case of the cooled heart. The heart was placed in a porcelain capsule and cooled or warmed by being surrounded by ice or with water at 40° C x denotes instant of stimulation.

we know, exceeds any value that has been observed for the Frog's heart, in which the latent period is usually between .1 and .2 sec.

The above remarks are applicable to the ventricular beat. We have also made one or two observations on the auricular beat with analogous results, viz., prolongation of the latent period and of the duration of contraction.

That lowered temperature is the chief factor in the prolongation of the latent period as in that of the duration of the contraction, we learned by experimentally varying the temperature, alternately cooling and warming the heart.

The effects as regards the latent period are especially striking; the same heart may respond to a stimulus only after a period of one second or more while it is in a relatively cold medium (12° to 0° C.), and when this is replaced by a relatively warm medium (38° to 40° C.) response becomes almost immediate.*

* We use this indefinite expression because our tracings were taken on a slow-rate cylinder, and
MDOOCLXXXVII.—B. 2 G

§ V. *The Wave of Contraction.*

It is not known whether or no there is any wave of contraction in the normal systole of the Mammalian heart, nor has it hitherto been sought for in the excised organ. There are no data to show whether, in the spontaneous or excited beat of the heart, its individual elements contract simultaneously or successively.

Our knowledge of the wave of contraction in the heart rests entirely upon observations made upon the excised organ of cold-blooded animals (Frog, Tortoise); the method employed has been to follow by rheotome and galvanometer the electromotive signs of an excitatory state at points more or less distant from a point of excitation. By this method, as applied by ENGELMANN, MARCHAND, and BURDON SANDERSON, the diphasic variation indicative of the passage of a wave of negativity has been plotted out, and its time-relations determined; the results thus obtained have been by BURDON SANDERSON controlled and confirmed by photo-electrometric records. The velocity of the wave thus determined has been given at 20 to 40 mm. per sec. (ENGELMANN), 100 mm. per sec. (MARCHAND), 125 mm. per sec. (B. SANDERSON and PAGE).^{*} These results apply to the excited beat of the "stanniused" Frog's heart. With regard to the spontaneous beat of the Frog's heart, a double variation indicative of negativity, first at base, then at apex, has been seen, and is properly received as evidence of the passage of a wave from base to apex in the spontaneous systole of the excised heart. No attempt has been published, as far as we know, to determine a wave of contraction in the beat of the Mammalian heart, whether spontaneous or excited, nor has the wave of contraction been recognised on the heart of any animal by a mechanical method analogous to that which AEBY[†] first applied to skeletal muscle.

We have examined the Mammalian heart for the phenomenon in question both by electrical and by mechanical methods. Deferring consideration of our results by the galvanometer and electrometer, we here briefly give the results of our application of a mechanical method, viz., application to the heart of a double myograph so as simultaneously to record the movements of its different parts. With regard to the instrument, we need only say that it consisted of two levers, 1 to 8 cm. apart, which were applied to the excised heart just as in AEBY's experiment they are applied to voluntary muscle. It will be well to give separately (a) the results we obtained upon the quiescent heart by excitation nearer to one or other of our levers; (b) the results we obtained on spontaneously beating hearts.

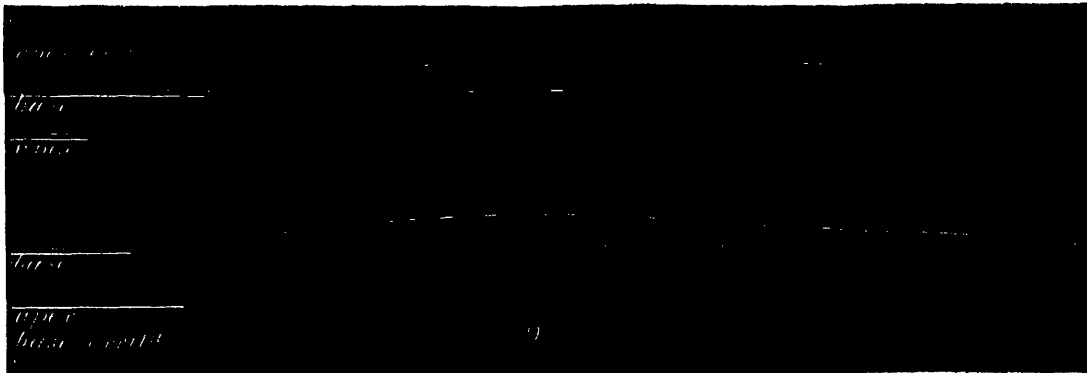
(a.) Excitation of a quiescent heart, upon which rested the two levers of our double cardiograph, at once revealed the fact that the total coordinated contraction of the

because we used mechanical stimuli. This was necessary, but did not give us the opportunity of taking fine measurements of time, which indeed we did not require.

^{*} ENGELMANN; PFLÜGER'S 'Archiv f. Physiol.,' vol 17; MARCHAND, *ibid.*, vol. 15 and 17; BURDON SANDERSON and PAGE, 'Journal of Physiology,' vol. 2, p. 384.

[†] AEBY, 'Archiv Anat. Physiol.,' 1860, p. 253.

Tracing 9.



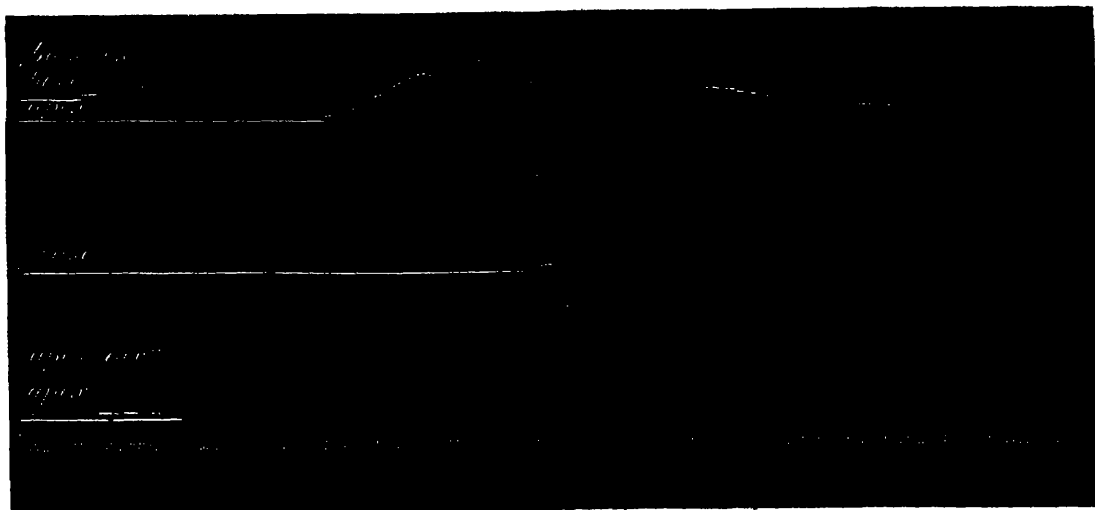
Wave of contraction in ventricle of Cat's heart, 30 minutes post mortem; taken by double cardiograph; mechanical stimulation near the base gives a ventricular contraction, in which the lever resting on the base commences to rise earlier than the lever resting near the apex; mechanical stimulation near the apex causes the lever near the apex to rise before the lever near the base.

Tracing 10.



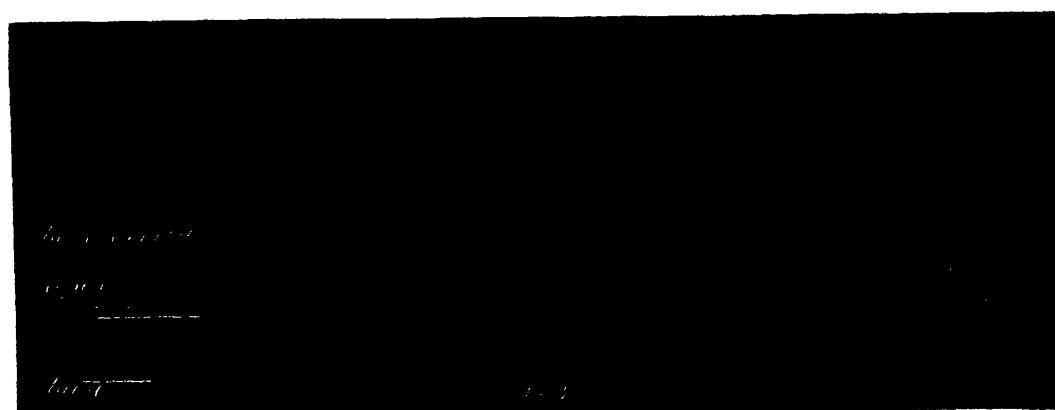
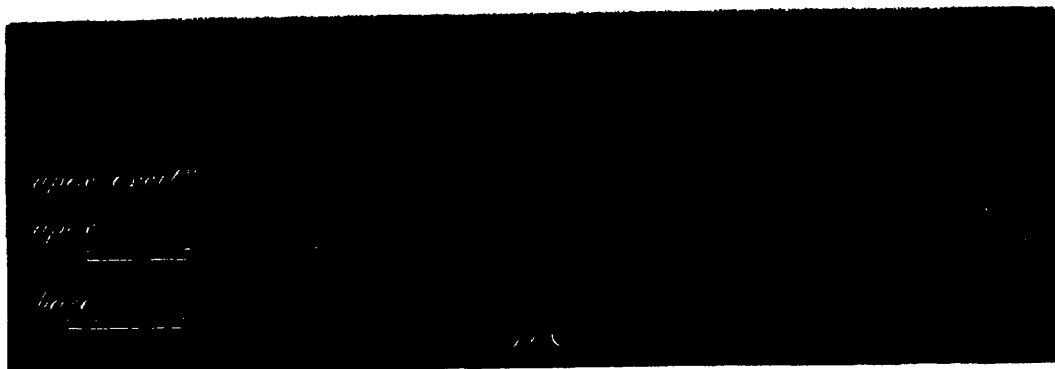
Wave of contraction in a strip cut obliquely from the right ventricle of a Dog, 10 minutes post mortem. Mechanical stimulation. The proximal lever is raised before the distal lever.

Tracing 11.



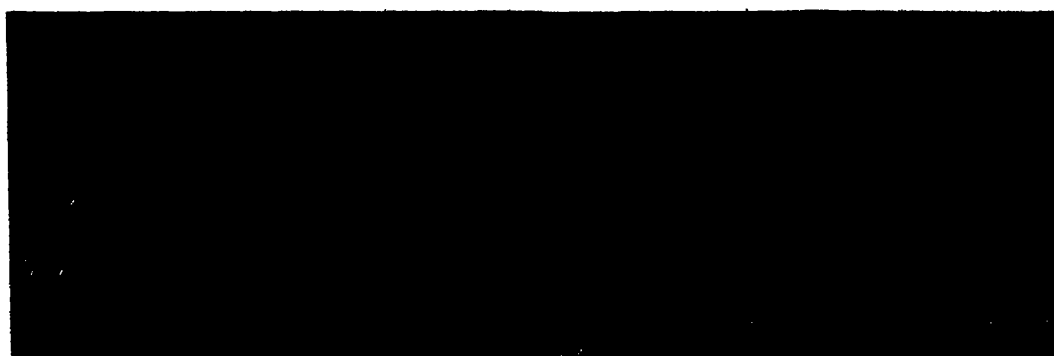
Wave of contraction in right auricle of Dog's heart. Upper line base, lower line apex. Excitation near base (β). Ditto. Excitation near apex (α).

Tracing 14.



Wave of contraction in Frog's ventricle. Upper line apex, lower line base. Excitation near apex (α).
Excitation near base (β).

Tracing 15.



Wave of contraction in spontaneous beat of Frog's heart taken by double cardiograph. The movement of the lever resting on the base precedes that of the lever resting on the apex. It is noteworthy that in all cases the contraction at the base outlasts that at the apex.

organ occurred as a wave starting from the point of excitation. The lever nearest to the excited point commenced to rise sooner than the more remote one, the difference being sometimes so considerable as to be plainly visible without record.

This, which is the fundamental fact, was clear and unmistakable in ventricle and in auricle, and we may anticipate upon a future section of our paper by adding that the galvanometric indication of the passage of a wave of negativity was equally clear and unmistakable in the form of a diphasic variation.

We thus learned that the wave known to occur in the excited beat of the "stanniused" Frog's heart occurs *under similar conditions* in the Mammalian heart, *i.e.*, in the excised quiescent organ. As may, however, be expected, the experimental shortcomings of the Mammalian heart are more frequent than in the case of the cold-blooded organ; complete and typically illustrative results are not obtained without fail, and variations are so great that a normal time-value of the wave cannot well be given.

But we have obtained records conclusively showing the existence of the wave in the ventricle and in the auricle, both in the entire organ and in strips of muscle cut therefrom. The experiments we have made in relation to this point are given in the accompanying *résumé* (Table F). Tracing 9 is an example of the wave of contraction in the left ventricle. Tracing 10 is an example of the wave of contraction in a strip of the right ventricle. Tracing 11 is an example of the wave in the right auricle of the Dog's heart. Table G contains the results of the few measurements we have made of this wave.

The results thus obtained on the Mammalian heart led us to apply the same method to the heart of the Frog; for this purpose we employed levers 5 mm. apart. As far as we knew, this had not yet been done: MARCHAND had attempted to obtain evidence concerning the rate of the contraction wave by alternately exciting the ventricle, near and far, from a part upon which a single lever had been adjusted; his attempt failed, as was to be expected, from the variability of the latent period. The double lever, by means of which the contractions of two parts near and far from a point of excitation are simultaneously recorded, avoided this source of error, and by it observations may be multiplied. Our measurements thus made give for the rapidity of the contraction wave in the Frog's ventricle, at between 8° and 12° C., a rapidity of between 30 mm. and 90 mm. per sec. These measurements are given at the end of Table F, and are illustrated by Tracing 14.

(b.) *The Spontaneous Beat.*—The evidence which we had obtained of the occurrence of contraction consequent upon excitation of the quiescent organ in the form of a wave starting from the excited point naturally led us to inquire whether or no a similar wave of contraction takes place in the normal spontaneous contraction. The testimony of the galvanometer is to the effect that such a wave does occur in the spontaneous beat of the excised Frog's heart; the rheotome is here inapplicable, but the double variation observed from the beating heart, when led off at base and apex, is such as to indicate the two phases: (1) negativity of base, (2) negativity of apex,

from which the conclusion is drawn that contraction proceeds from base to apex. With regard to the rate of propagation of the excitatory state in the spontaneous beat we have no information.

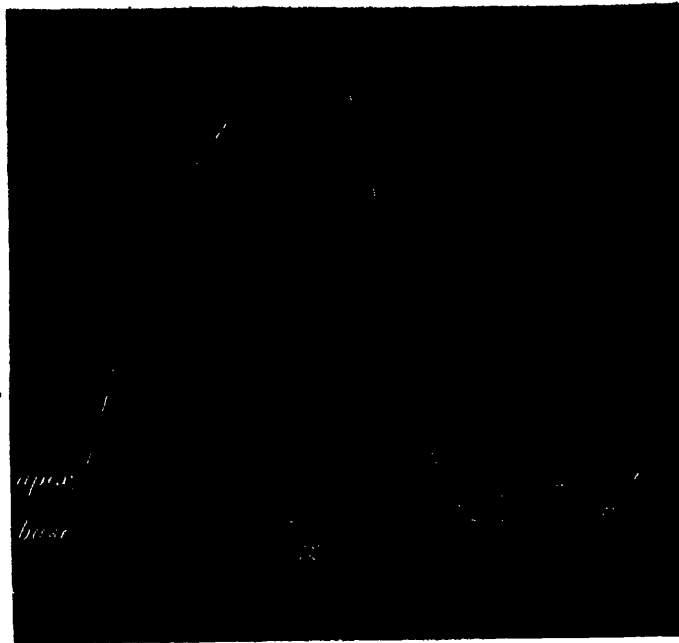
The application of two levers, 5 mm. apart, to the ventricle of the spontaneously beating Frog's heart at once shows that the contraction is from base to apex, and the difference in time between the rise of the lever nearer to the base and that of the lever nearer to the apex can be measured with reasonable accuracy; this time-difference shows that the wave of spontaneous contraction occurring from base to apex has a rapidity of about 100 mm. per sec. at a temperature of 9° C. (Tracing 15). As regards the spontaneous beat of the Mammalian heart, a similar method led us to results markedly different from those we had obtained in the Frog's heart. We have examined the hearts of Cats, Rabbits, Dogs, and Sheep, and find that in the spontaneous beat of the excised organ the contraction of the apex generally appears to precede that of the base; in some cases we have been unable to detect any difference between base and apex: in only two cases have we seen the contraction of the base precede that of the apex. The accompanying Table, G, summarises our results upon this point, and we give examples of the records we have taken in Tracings 12 and 13. It may not be superfluous to add that we have exercised all possible caution and care, paying due regard to correspondence of levers, pressure of pens, and comparative size of contractions.

It appears from our Table, firstly, that the rapidity of the wave is in general much greater in the hearts of warm-blooded animals than in that of the Frog. Secondly, that in Mammals it is more rapid in large hearts than in small; the maximum rapidity which we have accurately observed was in a Sheep's heart, the record being taken 4 minutes after death by bleeding, the levers being 8 cm. apart; the time-difference was .01 sec., giving a rapidity per sec. of 8 metres. Thirdly, the rapidity of the wave progressively decreases after excision of the heart.*

* When we made our observations we were not acquainted with those of F. KLUG (Du Bois-Reymond's 'Archiv für Physiologie,' 1881, p. 265, and 1883, p. 398). In his first paper he stated as the probable conclusion to be drawn from his observations that apex precedes base in the normal systole of the hearts of Frogs and of Rabbits. No records are given. His method of observation was, however, not free from objection, and in his second paper he states that the evidence derived from his previous observations is of no value. The observations were made upon hearts *in situ*, with intact circulation, so that the discharge of blood by the auricle caused a movement of the ventricle which prevented the determination, with a sufficiently rapidly travelling surface, of the commencing ventricular contractions.

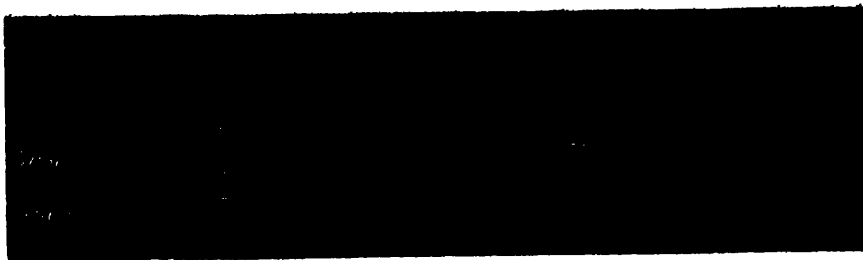
The opinions of HALLER, SENAC, ARNOLD, and of a British Association Committee in 1843 (quoted by KÜSCHNER in WAGNER's 'Handw. der Physiol.,' 1844, vol. 2, p. 85), were various; they were based on simple inspection of the heart's movements.

Tracing 12.



Wave (P) of contraction in ventricle of Cat's excised heart, beating spontaneously, 2 minutes post mortem. The vertical lines *r, r* indicate corresponding positions of levers, that resting on the apex being a little in advance of that resting on the base.

Tracing 13.



Spontaneous contraction of Cat's heart, recorded by double lever, 3 minutes post mortem. The movement of the lever resting on the apex precedes that of the lever resting on the base. The vertical lines *r, r* mark correspondence of levers.

§ VI. *Galvanometer Indications.*

It is necessary first to describe the plan of our apparatus, and the meaning of the abbreviations we shall have to use.



The heart, having been excised, was laid upon an insulating support, slightly hollowed to receive it, and the ventricle, right or left, led off by unpolarisable electrodes applied near base and near apex respectively. The disposition adopted was such that the apex was always in connection with the north screw of the galvanometer, the base being in connection with the south screw; we call these points respectively A and B. Current through the galvanometer from A to B is indicated by a deflection south of the magnet, current from B to A by a north deflection: it is thus apparent that a north deflection indicates that A becomes negative to B; that a south deflection indicates that B becomes negative to A. To denote the deflections north and south respectively, we use the letters N and S for large deflections, *n* and *s* for small deflections. We used a very delicate THOMPSON galvanometer ($R = 13,000$ ohms); the periodicity of its oscillations was such as not to allow us to make observations at intervals of less than half a minute.

This was in some cases a disadvantage; it was, however, soon apparent to us that the condition of the heart itself is generally such as to forbid the too frequent repetition of excitations.

In observations of the kind which we had to make it would have been useless to take exact measurements in number of degrees, and we contented ourselves with the occasional use of the qualifications "large" and "small" to express the relationship to each other of the two phases of the diphasic variation which usually came under our observation.

Any electrical inequality between the two led-off points was compensated in the usual manner by means of a SANDERSON'S potentiometer; we used the same instrument to take measurements of such differences of potential.

Our observations by the galvanometer are in the main confirmatory for the Mammalian heart of the fundamental facts established on the Frog's heart by the researches of ENGELMANN, BURDON SANDERSON, MARCHAND, and others, but with certain reservations and amid frequent irregularities, owing to the presumably more mobile nature of the Mammalian organ. We know already, from the observations of

KOLLIKER and MULLER,* that the spontaneous systole of the Mammalian heart is accompanied (or rather preceded) by an electromotive change; to this we have to add that the electromotive change is frequently diphasic, and entirely similar to the diphasic variation of the spontaneously beating Frog's heart. The second chief fact relating to spontaneous action is that electromotive changes, such as ordinarily belong to visible contraction, frequently persist in the absence of such visible contractions, and continue long after these have entirely ceased, *i.e.*, invisible molecular changes outlast visible changes of form.

(a.) *Excited contractions.*—As regards excited beats, one fundamental result is that excitation applied near to one of two points, by which any two parts of the excised, but otherwise uninjured and quiescent, heart are led off to the galvanometer, gives rise to a diphasic variation the direction of which is such as to indicate (1) negativity of the proximal electrode; (2) negativity of the distal electrode. These are the most important points which we have been able to satisfactorily establish. To these may be added a fourth statement, *viz.*, the effect of local injury long after the heart has become quiescent, *i.e.*, inexcitable, and is apparently dead, is to develop a local alteration of potential, the injured part becoming negative to all other parts.

The details of our experiments show many irregularities, some of which we can only partially understand, but which are doubtless attributable to irregularities and inequalities in the dying organ; these may have been due to differences of temperature or accidental injuries, or other spontaneously occurring inequalities of excitability at different points. It might be expected that an explanation of these should be found by the experimental establishment of such irregularities: we have sought for such an explanation and failed to find it; and we attribute this failure to the great susceptibility of the excised Mammalian heart to experimental interference. Our laboratory notes contain abundant examples of this extreme susceptibility to apparently trivial causes. To mention an example, we have frequently noticed that a slight touch with a blunt pointed instrument near one of our two electrodes was sufficient to develop *permanent* negativity at the part, indicative of a slight degree of injury. It consequently happened, as the most frequent exception to the classical effect, that instead of the diphasic variation, indicative of negativity at the first and second contacts respectively, we obtained only a simple variation. We think that the apparent local negativity developed under these conditions was in different cases attributable to either of two causes: (1) injury; (2) the excitatory state. The first cause is indicated by a single permanent deflection, the second by a single swing; and that this is an excitatory effect is indicated by the fact that the first variation consequent on excitation is often followed by a second or a third such variation, and that the series of single variations thus initiated may terminate with a typical diphasic variation. We have exceptionally noticed that local excitation might give rise to a single variation, indicating that the electrode furthest from the point of

* 'Würzburg, Phys. Med. Gesell. Verhandl.,' vol. 6, 1856, p. 529.

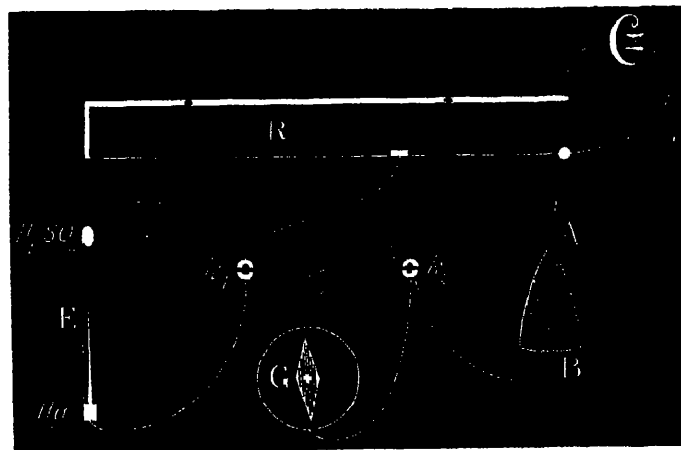
excitation becomes negative to the proximal electrode. This result is exceptional, and, although at first sight anomalous, can, we think, be reasonably explained. We think it is due to unequally excitable tissue at the two led-off points, and that the inequality consists in diminished excitability near the first lead-off in comparison with greater excitability near the second lead-off. Our notes contain several experiments, the analysis of which justifies this conclusion, and we may refer to Experiment No. 31 (Remarks on Galvanometer Experiments) as an instance in point.

We have never seen a diphasic variation such as to indicate negativity starting from the distal electrode. These results are in agreement with those previously described under § V. (a). We have usually applied our electrodes near the base and near the apex of the ventricle, both right and left; we have also applied them laterally to the right and left border of each ventricle. We have also examined the variations with an electrode on each ventricle, and with one electrode upon the auricle, the other on the ventricle. As regards all positions of the electrodes on the two ventricles, we have observed that the diphasic variation is the rule by excitation near either electrode. Our observations go to show that the ventricular portion of the excised Mammalian heart is an indifferent physiological conductor of the excited impulses in all directions. There are in the ventricular mass no indications of directions of greater or less resistance to the passage of the excitatory state. As regards the junction between auricle and ventricle, we have never seen any evidence of the passage of negativity in either direction. When an electrode is placed on either auricle and ventricle respectively of the same side, and excitation is applied to either cavity, the result has always been a single variation indicative of negativity of the electrode applied to it. We have never obtained a diphasic variation, nor have we ever observed a diphasic variation with electrodes on each auricle. These statements are based on experiments made after spontaneous contractions had ceased, viz., half to two hours after excision of the heart.

(b.) *Spontaneous contractions.*—As regards the electromotive changes with visible spontaneous beats, our results show no uniformity; we can find in them no evidence either for or against the results which we obtained by the graphic method. The direction of the deflection when the heart was connected with the galvanometer by base and apex was very variable, and indicated no regular origin or mode of progression of the excitatory process. We can say no more on this point than that out of 62 experiments we observed N in 17 cases, S in 17, NS in 16, and SN in 12. Under these circumstances, and in the anticipation that this difficult question may be further pursued, and the conditions of variety in results determined either by ourselves or by others, we think it best to place on record a tabular summary of our results (Table I.), in the hope that the data therein consigned may prove to be of further use. We intend to pursue this question in a future investigation.

§ VII. *Electrometer Indications.*

An examination by the electrometer of the electromotive changes, as revealed by the galvanometer, is of obvious importance. The capillary electrometer of LIPPMANN gives indications which follow rapid changes of potential far more faithfully than do the indications of the galvanometer, whether "dead-beat" or freely oscillating. We arranged our connections according to the following diagram :—



The heart is led off at A and B. By the two short circuiting keys, K_1 and K_2 , its current can be sent either through the electrometer E or through the galvanometer G. The current is compensated from the battery and rheochord R (its key is not represented in the diagram). The electrometer E is fixed on the stage of a microscope, the tube of which projects into a dark chamber, and the image of the field of the microscope, with the capillary column of mercury, is thrown upon a sheet of ground glass, on which its movements can be observed through an aperture in the dark chamber. The movements of the electrometer are recorded, when desired, by substituting a travelling sensitive surface for the ground glass, all light, with the exception of that passing through the capillary, being shut off from the plate by a screen in which a vertical slit is cut. Our photographs, except where otherwise stated, were taken with a $\frac{1}{2}$ -inch objective, and the image was formed 90 cm. behind it. We made use of sunlight for our photographs, reflected from the ordinary sub-stage mirror of the microscope.

The movements of the mercury in the capillary—advance and retreat—are in the same direction as the direction of current; hence, when A is negative to B, the movement of the mercury is northwards in the field of the microscope, and, the column being arranged to block the light focussed through the capillary on to the travelling sensitive surface, this movement appears on the photographic negative as a white projection into a darkened area; the reverse occurs if B becomes negative to A, and

a black projection into a light area is recorded on the negative. Of course this is again reversed in the positive prints of such negatives.*

We did not, as in the case of the galvanometer, adopt only one mode of connection of the electrometer, but varied it so that sometimes A was in connection with H_2SO_4 , sometimes with Hg; this we considered advisable, seeing that the movements of the capillary column do not take place with equal facility in both directions, but more easily towards than from the end of the tube. But, to preserve uniformity in the registration of our results, we give them as if the connection were throughout as in the diagram; negativity of A is then denoted by a north variation, negativity of B by a south variation (apparent variation in field of microscope).

(a.) *Of spontaneous contractions.*—Our first trials showed at once that the readings of the electrometer agree with those of the galvanometer, but that, as might be expected, rapid changes are read by the former instrument which escape observation by the latter. Thus, *e.g.*, in Experiment 42 the spontaneous variation galvanometrically was N, while electrometrically it was SN, and in Experiment 44 it was galvanometrically S, electrometrically NS. The superiority of the electrometer is not, however, fully made use of without having recourse to the photographic method, by which a permanent record of the variations is made. Whereas by the swinging magnet or swinging coil of a galvanometer magnitude and duration of electromotive changes, such as occur with action of the heart, are compounded and cannot be separated, their separate estimation by the electrometer is to some extent possible; how far this is so we do not know, doubtless the curve described by the mercury column does not give the absolute course as to duration and magnitude of a very rapid electromotive change; different electrometers, or the same electrometer at different times, may have a different amount of deforming effect. This is, however,

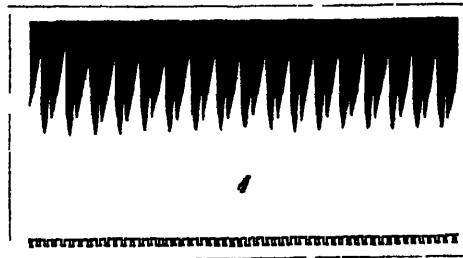
* The following key will be of service for the reading of our photographs :—

With A to H_2SO_4 and B to Hg (as in diagram).—If A becomes negative to B, the mercury moves towards the end of the capillary, *i.e.*, northwards in the field of the microscope; the photographic negative shows a light projection into a dark area, the positive shows a dark projection into a light area. If B becomes negative to A, all is reversed; the mercury moves southwards in the electrometer image, and the negative shows a dark projection into a light area, the positive shows a light projection into a dark area.

With A to Hg and B to H_2SO_4 .—If A becomes negative to B, the photographic negative shows a dark projection into a light area. If B becomes negative to A, the photographic negative shows a light projection into a dark area. In all our photographs the upper border of the figure corresponds with the north of the microscope field; the time-tracing is recorded at this border. In the woodcuts, which are the reproduction of photographic positive prints, the lower border of the figure corresponds with the north of the microscopic field; the time-tracing is recorded at this border. The black portion of the woodcuts reproduces blocking of light by the mercury column, but in the woodcuts North movement of the mercury is represented by black projection towards the time-tracing, South movement by white projection in the opposite direction. All photographs read from left to right. The rates of movement of the sensitive surface are recorded on each plate. Their actual values are given with photos. 3 and 4.

out of our control: we can only submit to the criticism of our readers examples, the data of which we have obtained by its means. All these data have been furnished by the same capillary tube. The review of these examples shows (1) that our instrument was perfectly capable of giving the record of diphasic variations,* (2) that in some cases only a monophasic variation is revealed. Our opinion is that Fact 1 is sufficient argument for accepting Fact 2 as a true indication of a single phase, and not regarding it as the compound indication of two phases. Our experiments showed us

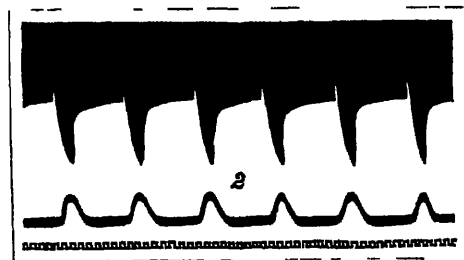
Photograph 1.



Frog's heart spontaneously beating, showing auricular variation, followed by ventricular.

NOTE.—All photographs read from left to right. The rates of movement of the photographic plate are indicated on the plates themselves. Two rates were usually employed, the slower of about 1 minute = .44 second, the quicker of about 1 minute = .08 second.

Photograph 2



Shows double variation of spontaneously-beating Frog's ventricle.

The electrical phases are in opposite direction at beginning and at end of beat; first phase, base negative to apex; second phase, apex negative to base.

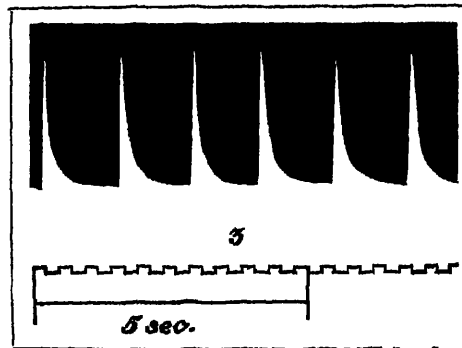
A simultaneous record is made of the movements of a lever resting on the ventricle. (Apex to H_2SO_4 , variation SN.)

that a double variation seen by the galvanometer is also seen and recorded to be double by the electrometer (Photos. 1 and 2); they showed further, as we expected, that a variation seen as simple with the galvanometer could be shown to be composed of two phases when the electrometer was put in circuit instead of the galvanometer. The fact, however, for which our previous experiments had not prepared us, was that

* We also obtained the diphasic variation of a single twitch of the Frog's gastrocnemius excited by an induction shock.

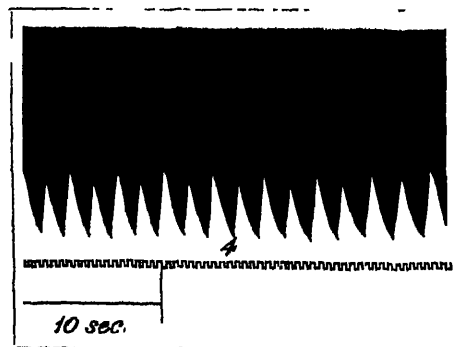
the electromotive change accompanying the beat of the ventricle is not always composed of two phases, it is sometimes expressed in its photograph as a single variation (Photos. 3 and 4). This then is the fact; our opinion concerning it is that, having regard to the rapidity with which the instrument may be shown to act to very brief changes in very rapid succession, the single variation observed under these circumstances is proof of a practically single and simultaneous change taking place

Photograph 3.



Spontaneous variations of Rabbit's ventricle immediately after excision. These are in this case monophasic, and such as to indicate that the apex became negative to the base at each beat. (Apex to Hg. Variation N.)

Photograph 4.

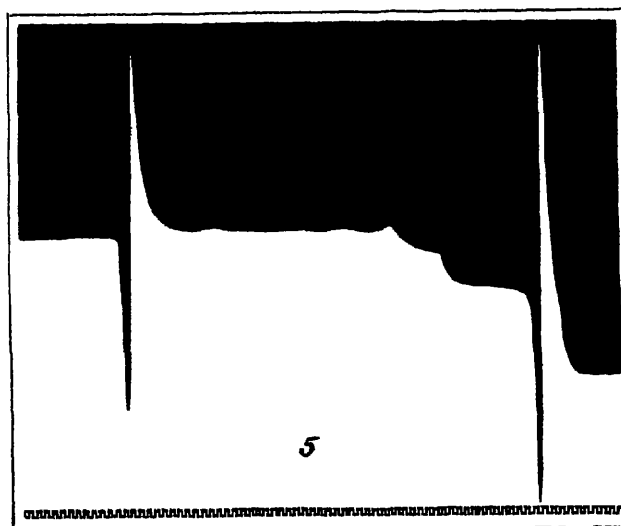


Spontaneous variations of Cat's ventricle. These are monophasic, and indicate that the base becomes negative to the apex. The electromotive variations show dirotism which corresponded with the dirotism of visible beats. Time post mortem 5 minutes. (Apex to H_2SO_4 . Variation S.)

throughout the ventricle, and disproof, or at least failure of proof, of the passage of a wave of excitation in the contractile substance. Our previous experiments by mechanical and by galvanometric methods had furnished the demonstration under certain conditions of the passage of a wave of excitation and of contraction at rates slow enough to be measurable, and the demonstration is confirmed by the electrometer (*diphasic variation*, Photo. 5). But our previous experiments by mechanical

and by galvanometric methods had in many cases failed to furnish this demonstration, and the failure is repeated by the electrometer (*monophasic variation*). The conclusion we draw from this is that in such cases the ventricles contract in their several parts with a synchronism such that no evidence of wave as regards the excitatory process is obtainable by any method we have used. Whether the synchronism be absolute or not, we cannot say; it does not affect the conclusion we are about to draw, viz., that the excitatory change must, under such conditions, be practically

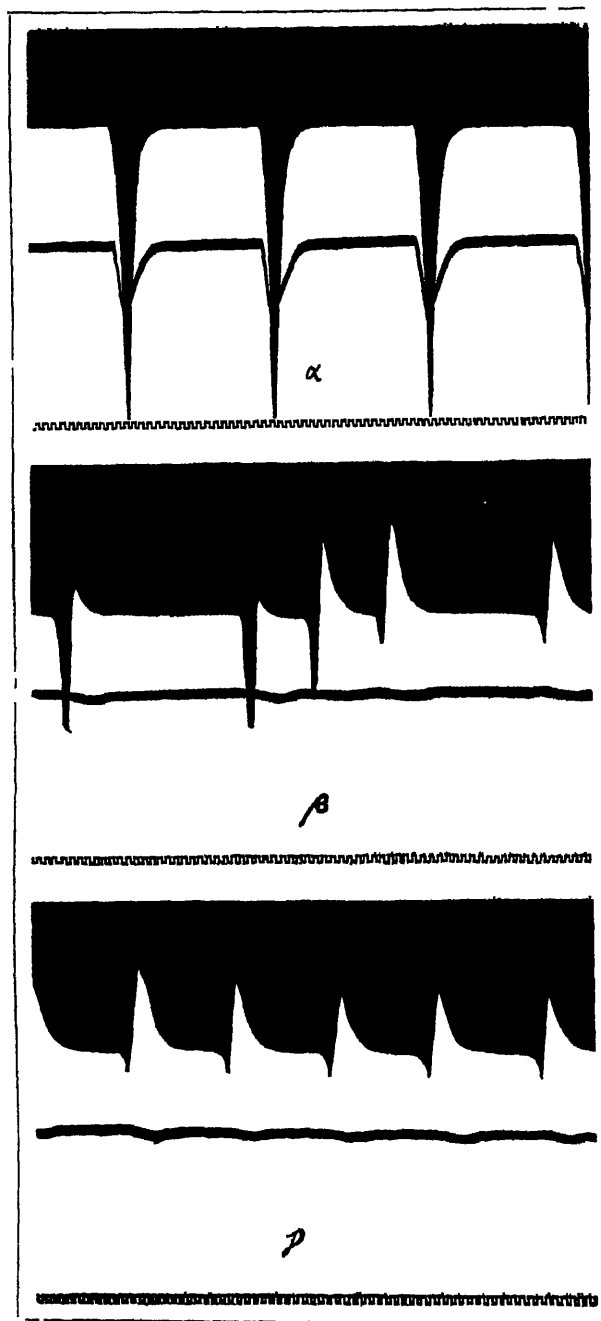
Photograph 5.



Puppy. Two double variations in ventricle. The first is of a spontaneous contraction and indicates: first phase, apex negative; second phase, base negative; the second variation is caused by mechanical excitation of the apex, and also shows first phase apex negative, second phase base negative. The mechanical excitations to produce this contraction were three in number; the first two were ineffectual, i.e., produced no visible contraction, they are, however, visible upon the tracing, the apex negativity having been permanently increased by each excitation. Apex negativity remained permanently increased after the diphasic variation had come to an end. (Apex to H_2SO_4 . Variation NS.)

simultaneous in all parts of the ventricle, and that such simultaneity postulates the existence of nervous channels of conduction. What are the conditions of the difference in the two cases? To this question we can only answer that we have, in general, observed the *monophasic variation during early moments after excision of the heart, the diphasic variation subsequently to these earlier moments*; more definite expressions we cannot use on account of the differences of individual hearts. We have further observed more than once the diphasic variation to supervene upon the monophasic in one and the same heart, as time advanced, without any alteration in the position of the electrodes (Photo. 6). As time advances, however, a diphasic may give place to a *late* monophasic variation, which is, to our mind, suggestive of a

Photograph 6.



A series of three photographs of variations of a Dog's heart taken without moving the electrodes.

a. Five minutes after death. The variation is monophasic, indicating negativity of apex.

β. Forty-five minutes after death. The variation is becoming diphasic; first phase apex negative to base; second phase base negative to apex.

In some of the variations the first phase is much more marked than the second, in others the second phase is the more marked.

γ. Sixty minutes after death. The variation has become uniformly diphasic, first phase apex negative to base; second phase base negative to apex, and the second phase is more marked than the first (Apex H_2SO_4 . Variation N and NS.)

different cause ; whereas we regard the early monophasic variation as the expression of local predominance of a change taking place throughout the whole ventricle, we think that the late monophasic variation is in reality due to local activity at and near one of the two leading-off electrodes ; this, indeed, is often demonstrable at this period by local excitation, which then causes contraction restricted to definite spots.

(b.) *Excited contraction*.—As regards the excitatory variation, our results with the electrometer were entirely similar to those which we have already described with the galvanometer. The accompanying Table summarises our electrometer observations. (Table K.)

§ VIII. *Conclusion.*

It would be superfluous to repeat, in summary, the facts relating to the mode of decline of the excised Mammalian heart which are described in the second, third, and fourth paragraphs of our paper. The extraordinary sluggishness of action, of which these showed us that the Mammalian heart is capable under the conditions of our experiments, tempted us to analyse as far as possible the mode and mechanism of cardiac contraction in Mammalia.

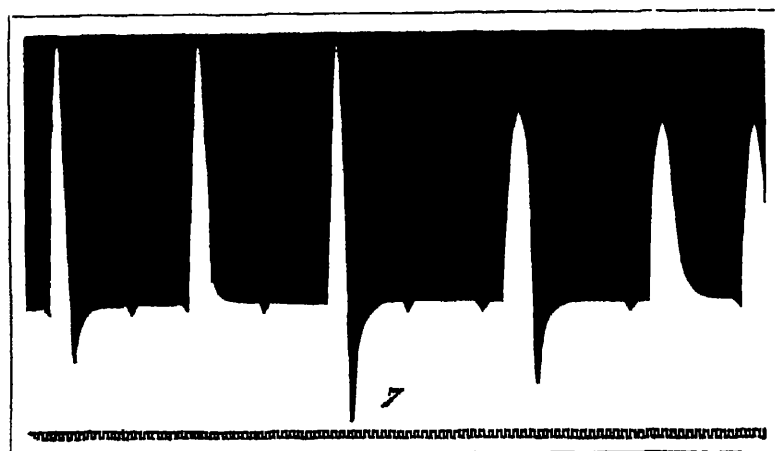
In this direction the chief result, which we regard as established, is that on the Mammalian heart, under conditions similar to those in which the Frog's heart has been examined in this respect, the passage of an excitatory wave is demonstrable as a diphasic variation with the galvanometer and the electrometer, and that the passage of a corresponding wave of contraction is demonstrable by mechanical methods.

As regards the Mammalian heart, neither of these facts have, so far as is known to us, been hitherto observed ; as regards the Frog's heart, we have incidentally added to the classical phenomenon of the diphasic variation a demonstration of its mechanical aspect as a wave of muscular contraction. The similarity between the excised Mammalian heart and the excised Batrachian heart is thus far complete. But there are points of divergence. Analysis of the spontaneous beat both by mechanical and by electrical methods brings these into evidence. The diphasic variation of the spontaneously beating Frog's heart is uniformly indicative of negativity of base, followed by negativity of apex ; to this we add the statement that muscular contraction of base precedes, and is followed by, muscular contraction of apex. All our experiments and measurements are thus confirmatory of the doctrine that the contraction takes place from base to apex, and that the wave of excitation and of contraction takes place by muscular channels. It is otherwise with regard to the Mammal heart. The variation is not always diphasic ; immediately after excision it is more frequently monophasic and thereafter becomes diphasic. The movement of galvanometer and electrometer indicates for the monophasic variation negativity predominant either at apex or at base ; for the diphasic variation (when it is present) negativity of apex, followed by negativity of base, or the reverse. There is no constant rule, in fact, and we saw no use in further multiplying observations, seeing that we had to do with the irregulari-

ties due to an excessive susceptibility to local injury; of such susceptibility we had abundant evidence; the delirium, which is so apt to supervene, is its extreme instance.

We see no escape from the conclusion that the Mammalian ventricle is an organ not only controlled by nerves, but also co-ordinated as to the action of its several parts through intra-muscular nervous channels. A monophasic variation can consist only with simultaneity of action throughout the organ, or with the successive action of its several parts so rapid as not to be revealed by either galvanometer or electrometer, or with action confined to one part of it, or with action predominant at one part; it is not consistent with the comparatively slow successive action of various parts of the

Photograph 7.



Variations accompanying spontaneous beats of Puppy's heart. The variations of the auricle are visible as small black teeth on the tracing, and it may further be seen that the variations of the ventricle are in two instances monophasic, indicating negativity of apex, and in three instances diphasic, with first phase = apex negative to base, second phase = base negative to apex. (Apex to Hg. Variation of auricle S; of ventricle N and NS.)

ventricle by muscular transmission. Such simultaneity or approximate simultaneity can, we think, only be effected by nervous channels, and that conduction by nervous channels plays a part in the simultaneous and co-ordinated action constituting a beat is supported by other considerations, by measurements of the rate of conduction, for instance, such as we have given above, and still more strongly, we think, by the electrometer sequence repeatedly observed by us when the heart is dying and its parts becoming evidently asynchronous in their action, viz., negativity of auricle, followed by negativity of apex, followed by negativity of base. This can consist only with the existence of nervous channels along which excitatory impulses have passed from auricle to the apex. (Photo. 7.)

Usually it is not till several minutes have elapsed after excision that the diphasic variation is seen. It doubtless depends on the fact that with the slowness of action asynchronism of acting parts becomes manifest, but whether we have to do with a

much retarded transmission from part to part along nerves or with muscular conduction is difficult to say with certainty; the entire similarity of the wave with a muscular wave is, however, sufficiently obvious. Finally, when transmission can no longer be effected, the heart responds to local excitation by a monophasic variation which may be temporary—indicative of local discharge of action,—or permanent—indicative of injury of tissue.

No portion of our inquiry gave us more trouble and doubt than the results of the mechanical exploration at base and apex of the spontaneously beating heart. Almost without exception we obtained apparently movement at apex antecedent to movement at base. All possible care was exercised, and we have given our results. We have been careful to say no more than that "the movement of the lever resting near the apex precedes that of the lever resting near the base."

Sometimes the contraction of the apex was visibly antecedent to contraction at the base—exceptionally, the precedence was with the base. In the case of the Frog's heart this was always the case.

A desire to obtain the variation of the absolutely normal and unexposed heart led us to the exploration of the human subject with the following result, viz., auricular followed by ventricular negativity anteceding respectively the auricular and ventricular events. We could obtain no evidence of a diphasic ventricular variation, and we have yet to exclude the possibility of the observed variation being caused by alteration of contact by the heart's impulse.*

* I have since ascertained that the variation on Man is in reality due to an action current, and not to alteration of contact. The variation precedes the cardiac impulse; it may also be observed when the hand and foot are dipped into two vessels of salt solution connected with the electrometer. Under these circumstances the variation is still observed preceding the cardiac impulse.—A. W., July 5, 1887.

TABLE A.—Cat's Heart. November 30, 1885. Decapitated 3.2 P.M.
Record starts 3.3 P.M.

| Time P.M. | Number of beats. | Duration. | Time P.M. | Number of beats. | Duration. |
|---|------------------|-----------|----------------------------------|--------------------------------|---------------------------|
| minutes. | | seconds. | minutes | | seconds. |
| 1 - 1½ | 20 5 | 2 | 15½ - 16 | 0 | |
| 1½ - 1¾ | 17 5 | 3 | 16 - 16½ | 2 | 2 55 |
| 1¾ - 1½ | 16 | 37 | 16½ - 16¾ | 1 | 2 7 |
| 1½ - 2 | 16 | 45 | 16¾ - 16½ | 2 | 2 85 |
| 2½ - 2¾ | 19½ | 67 | 17 - 17½ | 1 | 3 |
| 2¾ - 2½ | 19½ | 82 | 17½ - 17¾ | 1 | 3 |
| 2½ - 3 | 12 | 9 | 17¾ - 17½ | 1 | 3 |
| 3 - 3½ | 10 | 1 | 17½ - 18 | 1 | 3 |
| 3½ - 3¾ | 7½ | 1 2 | 18½ - 18½ | 1 | 2 85 |
| 3¾ - 4 | 8½ | 1 | 18½ - 18¾ | 1 | 3 15 |
| 4 - 4½ | 7 | 1 27 | 18¾ - 19 | 1 | 3 |
| 4½ - 4¾ | 7 | 1 27 | 19 - 19½ | 2 | 3 15 |
| Time lost in changing cylinders, ¼ minute. | | | 19½ - 19¾ | 1 | 3 3 |
| | | | 19¾ - 20 | 1 | 3 3 |
| | | | 20 - 20½ | 5 } delirium | Beats 6 to 12 second |
| | | | 20½ - 20¾ | | |
| | | | 20¾ - 21 | { 5 (subsequent to delirium) } | |
| 5½ - 5¾ | 5 | 1 5 | 20¾ - 21 | | 1 5, 1 8, and 2 2 seconds |
| 5¾ - 6 | 4½ | 1 5 | 21 - 21½ | 1 | 3 75 |
| 6 - 6½ | 4½ | 1 65 | 21½ - 21¾ | 1 | 3 75 |
| 6½ - 6¾ | 4 | 1 8 | 21¾ - 21½ | 0 | |
| 6¾ - 7 | 4 | 1 8 | 22 - 22½ | 1 | 4 5 |
| 7 - 7½ | 3 | 1 95 | 22½ - 22¾ | 0 | |
| 7½ - 7¾ | 3 | 2 1 | 22¾ - 22½ | 0 | |
| 7¾ - 8 | 3½ | 2 1 | 22½ - 23 | 1 | 4 5 |
| 8 - 8½ | 2 | 2 1 | 23½ - 23½ | 2 | 3 |
| 8½ - 8¾ | 3 | 2 17 | 23½ - 23¾ | 1 | 4 5 |
| 8¾ - 8½ | 2½ | 2 25 | 23¾ - 24 | 1 | 4 2 |
| 8½ - 9 | 2½ | 2 25 | 24 - 24½ | 0 | |
| 9½ - 9¾ | 3 | 2 25 | 24½ - 24½ | 2 | 4 5 |
| 9¾ - 9½ | 2 | 2 4 | 24½ - 25 | 0 | |
| 9½ - 10 | 2 | 2 4 | 25 - 25½ | 0 | |
| 10 - 10½ | 2 | 2 4 | 25½ - 25½ | 0 | |
| Time of changing cylinder (new paper cut and smoked), 5½ minutes. | | | Excitation commenced after this. | | |

TABLE B.—Cat's Heart. December 2, 1885.

| Minutes P M. | Number of beats. | Minutes P M. | Number of beats. |
|---------------------------------|------------------|-----------------------------------|------------------|
| $3\frac{1}{4}$ — $3\frac{1}{4}$ | 16 | $8\frac{1}{4}$ — $9\frac{1}{4}$ | 12 |
| $3\frac{1}{4}$ — $3\frac{3}{4}$ | 15 | $9\frac{1}{4}$ — $10\frac{1}{4}$ | 9 |
| $3\frac{3}{4}$ — 4 | 14 | $10\frac{3}{4}$ — $11\frac{3}{4}$ | 7 |
| 4 — $4\frac{1}{4}$ | 13 | 12 — 13 | 6 |
| $4\frac{1}{4}$ — $4\frac{3}{4}$ | 12 | $13\frac{1}{4}$ — $14\frac{1}{4}$ | 5 |
| $4\frac{3}{4}$ — 5 | 11 | $14\frac{1}{4}$ — $15\frac{1}{4}$ | 4 |
| 5 — $5\frac{1}{4}$ | 9 | $15\frac{3}{4}$ — $16\frac{3}{4}$ | 4 |
| $5\frac{1}{4}$ — $5\frac{3}{4}$ | 9 | 17 — 18 | 3 |
| $5\frac{3}{4}$ — 6 | $7\frac{1}{2}$ | $18\frac{1}{4}$ — $19\frac{1}{4}$ | 3 |
| 6 — $6\frac{1}{4}$ | 7 | $19\frac{1}{2}$ — $20\frac{1}{2}$ | 2 |
| $6\frac{1}{4}$ — $6\frac{3}{4}$ | 6 | $20\frac{3}{4}$ — $21\frac{3}{4}$ | 1 |
| $6\frac{3}{4}$ — $6\frac{1}{2}$ | 6 | 22 — 23 | 1 |
| 7 — $7\frac{1}{4}$ | 5 | | |
| $7\frac{1}{4}$ — $7\frac{1}{2}$ | 4 | | |
| $7\frac{1}{2}$ — $7\frac{3}{4}$ | 4 | | |
| $7\frac{3}{4}$ — 8 | 4 | | |

TABLE C.—Table showing Beneficial Effect of Pause.

| Number of the contraction. | Height. | Interval. | Number of the contraction. | Height. | Interval. |
|----------------------------|----------|-----------|----------------------------|----------|-----------|
| | millims. | seconds. | | millims. | seconds. |
| 1 | 25 | 9 | 14 | 13 | 18.75 |
| 2 | 14 | 10.8 | 15 | 17.5 | 19 |
| 3 | 19 | 13.5 | 16 | 14 | 4.3 |
| 4 | 16.5 | 12.3 | 17 | 9 | 30.3 |
| 5 | 17 | 16.5 | 18 | 20 | 140.21 |
| 6 | 17 | 17.5 | 19 | 16.5 | 84.15 |
| 7 | 17 | 16.2 | 20 | 17 | 21.60 |
| 8 | 16 | 29.25 | 21 | 14 | 7.5 |
| 9 | 22 | 30.9 | 22 | 9.5 | 60.9 |
| 10 | 18.5 | 70.5 | 23 | 17 | 238.2 |
| 11 | 21 | 100.65 | 24 | 15 | 11.25 |
| 12 | 19 | 6.45 | 25 | 9 | |
| 13 | 9.5 | 5.25 | | | |

TABLE D.—Rabbit. December 4, 1885. Auriculo-ventricular Block.

| Minutes P.M. | |
|--|---------------------------------------|
| 3- 6 | All auricular contractions pass over. |
| 6-12 | Every other one passes over. |
| 12-14 | Every 3rd-4th passes over. |
| 14-15 | Every 4th-5th passes over. |
| 15-16 | Every 8th-10th passes over. |
| 17-18 | Every 8th-10th passes over. |
| 18-19 | Every 16th passes over. |
| 20 | Every 16th passes over. |
| After 22 minutes none get over. | |
| The block increases very rapidly after about 14 minutes P.M. | |

TABLE E.—Table of Latencies. (Mammalian Hearts.)

| Date. | Animal. | Time P.M. | Latent time. | Length of contraction. |
|----------------|--------------|---------------|-----------------|------------------------|
| | | minutes. | seconds. | seconds |
| 1885. | | | | |
| Nov. 27 . . . | Rabbit . . . | 11 | .25 | |
| Nov. 28 . . . | Rabbit . . . | 60.5 | .45 | |
| " . . . | " . . . | 62 | .45 | |
| " . . . | " . . . | 63.5 | .49 | |
| " . . . | " . . . | 65 | .45 | |
| " . . . | " . . . | 66.5 | .6 | |
| " . . . | " . . . | 68 | .45 | |
| " . . . | " . . . | 69.5 | .6 | 6 |
| Nov. 30 . . . | Cat . . . | 25.5 | .3 | |
| " . . . | " . . . | 27 | .45 | 4 |
| " . . . | " . . . | 30 | .6 | |
| Dec. 4 . . . | Cat . . . | 27.5 | .27 | 4 |
| " . . . | " . . . | 32 | .45 | 9 |
| Dec. 4 . . . | Rabbit . . . | Later than 45 | .5, .6, and .87 | 5 |
| Dec. 5 . . . | Kitten . . . | 11.5 | 2. | |
| Dec. 5 . . . | Rabbit . . . | 3 | .15 | |
| " . . . | " . . . | 3.5 | .225 | |
| " . . . | " . . . | 4 | .37 | |
| Dec. 8 . . . | Cat . . . | 30 | .37 | 3.7 |
| " . . . | " . . . | Later | .5 | |
| " . . . | " . . . | " | .5 | |
| " . . . | " . . . | " | .85 | |
| " . . . | " . . . | " | .775 | |
| " . . . | " . . . | " | 1.075 | |
| " . . . | " . . . | 95 | 1.725 | 5 |
| Dec. 9 . . . | Rabbit . . . | 8.5 | .25 | 2.5 |
| " . . . | " . . . | 9.5 | .25 | |
| " . . . | " . . . | 11 | .275 | |
| Dec. 12 . . . | Rabbit . . . | 10 | .2 | 2.5 |
| Dec. 14 . . . | Cat . . . | 26.5 | .6 and .9 | 4.5 |
| " . . . | " . . . | 31.5 | 2. | |
| 1886. | | | | |
| Jan. 1 . . . | Kitten . . . | 11 | .175 | 2 |
| " . . . | " . . . | Later | .2 | |
| " . . . | " . . . | " | .225 | |
| " . . . | " . . . | " | .25 | |
| " . . . | " . . . | 21 | .35 | 3.75 |
| " . . . | " . . . | 22 | .5 | |
| Jan. 2 . . . | Cat . . . | 10 | .1 | .6 |
| Jan. 7 . . . | Cat . . . | 18 | .275 | 2.75 |
| " . . . | " . . . | 19 | .325 | 3.12 |
| " . . . | " . . . | 23 | .57 | 3.75 |
| " . . . | " . . . | 24 | .625 | 5 |
| Jan. 22 . . . | Cat . . . | 3 | .150 | 1.62 |
| " . . . | " . . . | 4 | .15 | 1.87 |
| " . . . | " . . . | 10 | .225 | 2.25 |
| " . . . | " . . . | 11 | .3 | 3 |
| " . . . | " . . . | 12 | .3 | 1.5 |
| " . . . | " . . . | 12 | .375 | 3 |
| Feb. 5 . . . | Cat . . . | 12 | .4 | 4 |
| " . . . | " . . . | 17 | .5 | 5.5 |
| Feb. 6 . . . | Dog . . . | 29 | .44 | |
| " . . . | " . . . | 28 | .40 | 1.4 |
| Feb. 9 . . . | Sheep . . . | 19 | .08 | .8 |
| March 1 . . . | Rabbit . . . | 10 | .225 | 2.25 |
| March 15 . . . | Dog . . . | | .22 | 2 |

TABLE F.—Rate of Wave in Excited Beats of Ventricles.

| Animal. | Time after death. | Rate of wave per second. | Remarks. |
|------------------|-------------------|-----------------------------|---|
| | minutes. | mma. | |
| Cat | .. | 40 | <i>Vide</i> tracing 9. |
| Rabbit | 5 | 364 | |
| Kitten | 11 | ∞ | |
| " | 21 | 1200 | |
| Cat | .. | 1200 | <i>Vide</i> tracing. From base to apex. From apex to base. (Latency = .4 sec.) From apex to base. From apex to base. From base to apex. Strip of right ventricle. " " " " " " " " " " <i>Vide</i> tracing 10. " " " " |
| Dog | 2 min. 30 sec. | 857 | |
| Cat | 14 | 272 | |
| " | 12 | 218 | |
| " | 18 | 54 | |
| Dog | 10 | ∞ | |
| " | 9 | 295 | |
| " | 10 | 369 | |
| " | 12 | 378 | |
| " | 13 | 284 | |
| " | 14 | 252 | |
| " | 24 | 142 | |
| " | 26 | 75 | |
| " | 10 | 367 | |
| " | 12 | 312 | |
| " | 22 | 189 | |
| Frog | .. | 70 | <i>Vide</i> tracing 14. |
| " | .. | 91 | |
| " | .. | 76 | |
| " | .. | 30 | |
| " | .. | 44 | |
| " | .. | 88 | |

TABLE G.—Wave of Contraction in Right Auricle of Dog.

| Time P.M. | Excitation. | Rate of wave per second. | Length of contraction. | |
|-----------|-------------|-----------------------------|---------------------------|------------------------------------|
| minutes. | | mma. | seconds. | |
| 33 | B | 45 | 2 | |
| 35 | A | 32 | 2.86 | |
| 36 | B | 26 | 2.3 | |
| 37.5 | A | 24 | 2.3 | |
| 60 | A | 14 | 4 | { Tracing 11α. Dog Temp. 12° C. |
| 5 | B | 25 | .. | Cat. |
| 41 | B | 18 | 2 | Tracing 11β. Dog. |

TABLE H.—Rate of Wave in Spontaneous Beats. Mammal.

| Animal. | Time P.M. | Rate of wave per second. | Lever rising first. | Remarks. |
|--|-------------------|-----------------------------|------------------------|---|
| | minutes. | mms. | | |
| Rabbit . . . | 4 | 450 | A | A > B |
| " . . . | 4-13 | ∞ | = | A slightly precedes B, where A > B. |
| " . . . | 15 | 1395 | A | A > B. |
| Cat . . . | 3 | 1000 | A | A = B. Tracing 13. |
| " . . . | 3 | 2400 | A | A > B. |
| Rabbit . . . | 3 | 1000 | A | A > B. |
| Cat . . . | 2, 4, 5, and 6 | ∞ | = | A = B. |
| Kitten . . . | 2, 3, 4, 5, and 6 | ∞ | = | A = B. |
| Cat . . . | 5 | 1200 | A | A > B. |
| " . . . | .. | ∞ | = | A = B. |
| " . . . | 2 | 400 | A | A > B. |
| " . . . | 4 | ∞ | = | A = B. |
| " . . . | 4-5 | ∞ | = | A > B. |
| Dog . . . | 1 | 1200 | A | A < B. |
| Cat . . . | 2 | 2400 | A | A > B. Tracing 12. |
| " . . . | 9 | 444 | A | A < B. |
| " . . . | 4 | 300 | A | A < B. |
| " . . . | 4 | 600 | A | A < B. |
| Dog . . . | 4 | 2400 | A | |
| Sheep . . . | 4 | 8000 | A | A > B. Duration of contraction .12 sec. |
| Dog . . . | 3-4 | 2320 | A | A > B. Duration of contraction 36 sec. |
| Guinea-pig . | 2-5 | 900 | A | A > B. |
| Cat . . . | 4-5 | 400 | B | A > B. |
| Kitten . . . | 5 | 300 | B | A = B. |
| Cat . . . | 5 | 1875 | A | A > B. |
| Rate of Wave in Spontaneous Beats. Frog. | | | | |
| | | 76 | B | |
| | | 35 | B | |
| | | 40 | B | |
| | | 20 | B | Tracing 15. |
| | | 12 | B | |
| | | 90 | B | |
| | | 100 | B | |
| | | 100 | B | |
| | | 100 | B | |
| | | 79 | B | |
| | | 133 | B | |

NOTE. $\left. \begin{array}{l} A > B \\ A = B \\ A < B \end{array} \right\}$ Signify that the curve of the apex lever was higher than, equal to, or less than that of the base lever.

TABLE I.—Abstract of Galvanometer Experiments.

| Experiment. | Date. | Animal. | P.V. | Spontaneous beats. | Apex excitation. | Base excitation. | Demarcation. | Time P.M. of registered demarcation. |
|-------------|-------------------|----------|------------|--------------------|------------------|------------------|--------------|--------------------------------------|
| 1 | 1885 Oct. 31 . | Cat . . | 2 hrs. 15' | N | | | | |
| 2 | Nov. 30 . | Cat . . | 1 hr. 30' | N | | | N '002 D | 90' |
| 3 | Dec. 2 . | Cat . . | 1 hr. 15' | .. | N | S | '0032 D | 53' |
| 4 | Dec. 3 . | Cat . . | 45' | .. | NS | S | S | 45' |
| 5 | Dec. 4 . | Rabbit . | 1 hr. 15' | .. | .. | .. | N '0136 D | 60' |
| 6 | Dec. 4 . | Cat . . | | | | | | |
| 7 | Dec. 4 . | Rabbit . | | | | | | |
| 8 | Dec. 14 . | Cat . . | | N | N | N | S | |

REMARKS TO GALVANOMETER EXPERIMENTS OF TABLE I.

Experiment 1.—Our first observation was made upon the heart of a Cat, which had been killed by chloroform 2 hrs. 15 mins. previously; we were looking for the effects of injury upon its E.M.F., and observed spontaneous variations similar to the variations which accompany the normal rhythmic beat; the heart was motionless during these variations, which were such as to indicate negativity of apex.

Experiment 3.—This heart showed no spontaneous variations 53 mins. after death; beats had ceased to be visible 35 mins. post mortem. Excitation gave rise not merely to a single variation, but to a series of variations, indicating negativity of the excited part; we noticed this at the following periods post mortem: 1 hr. 18 mins., 1 hr. 53 mins., and 2 hrs. Thermal excitation of apex 2 hrs. 10 mins. post mortem gave S variation; at 2 hrs. 13 mins. post mortem excitation at base and apex gave S.

Experiment 4.—Electrode A was at apex of left ventricle; electrode B was at base of right ventricle.

Experiment 5.—The demarcation current was such as to indicate negativity at apex; it rapidly diminished from the moment when it was made.

1 hr. 10 mins post mortem the demarcation current was . . . '0056 D N

1 hr. 30 mins. post mortem '0032 D

Section at apex now caused it to rise to '0192 D

A few minutes later it stood at '0184 D

And a section at the base reduced it to '0128 D

The injury variation was thus considerably greater at apex than at base.

The excitatory variations were unsatisfactory, showing great want of uniformity, partly due, no doubt, to shifting of contacts.

Spontaneous variations were observed 1 hr. 20 mins. post mortem, there being at this time no trace of visible movement of the heart; they were subsequent to thermal excitation.

Experiment 6.—Cat's heart. 3 hrs. 21 mins. post mortem. Electrodes applied to base and apex were iso-electric; section of apex gave a N deflection of '0036 D. The entire heart was in rigor, and we could not satisfy ourselves that excitation had any effect.

Experiment 7.—Rabbit's heart. 5 hrs. post mortem. The left ventricle was in rigor, the right ventricle flaccid; electrodes on middle of right and left ventricle respectively showed no difference of potential.

Apex and base of right ventricle gave a current of '0011 D N. On left ventricle a current of '00016 D N.

Section of apex of left ventricle increased this to '001 D N.

Section of base converted it to '024 D S.

On the right ventricle section of apex increased current from '0011 D to '016 D N.

Experiment 8.—The demarcation current was such as to indicate negativity of base, viz., S; all variations spontaneous, excited from apex, or excited from base, gave a variation in the same sense, viz., N, which we could not attribute to shifting of contacts.

Section at base gave a small S deflection in comparison with a large N deflection caused by section at apex. It is allowable to conclude from the original direction of the demarcation current that the base was more injured than the apex at the commencement; this is confirmed by the effects of section at the two points, the effect being greater at the apex than at the base.

As regards the excitatory variations, the results are paradoxical, but intelligible on the supposition that the excitatory variation is manifested by the less injured part alone, viz., in this case the apex; and upon this supposition it is intelligible that the spontaneous beat should have given a similar variation.

TABLE I.—Abstract of Galvanometer Experiments—(continued).

| Experiment. | Date. | Animal. | P.M. | Spontaneous beats. | Apex excitation. | Base excitation. | Demarcation. | Time P.M. of registered demarcation. |
|-------------|--------------|----------|--|--------------------|------------------|------------------|-------------------|--------------------------------------|
| 9 | 1885 Dec. 14 | Kitten . | 0-1 hr. 30' | N | S | S | N before excision | 120' |
| 10 | Dec. 16 . | Kitten . | (After excision) .. | . | S | S | S .0344 D | |
| " | " | " | .. | N | N | N | N before excision | |
| " | " | " | (Position of electrodes altered) Later | S & nS | NS | SN | S .0005 | |
| 11 | Dec. 17 . | Kitten . | | SN | NS | S | Iso-electric | |

Experiment 9—Stimulus, with heart *in situ*, whether at base or at apex, gave S deflection; the demarcation current at the time showing apex to be most injured

After excision the demarcation current was reversed and equal to .0344 D S, and still excitation, whether at base or apex, gave S deflection.

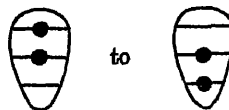
The first part of the experiment showed very clearly the persistence of spontaneous electromotive variations after the complete cessation of visible beats; the effects of excitation are paradoxical, and we cannot explain them.

Experiment 10.—Kitten heart in body almost iso-electric; apex slightly negative to base.

Heart excised. Demarcation .0005 D S.

Spontaneous variation; base and apex excitation alike gave N.

Position of electrodes was altered from



Spontaneous beats are now S and occasionally nS

Base excitation gave SN.

Apex excitation gave NS.

Later.—Base excitation gave S.

Apex excitation gave N, and injury near A gave permanent deflection N, injury near B permanent deflection S.

The first part of the experiment, *i.e.*, with the electrodes in the first position, was paradoxical, but intelligible upon a supposition similar to that made in case of Experiment 8, *viz.*, that the excitatory variation is pre-potent at the less injured part, in this case nearer to the apex.

The effects observed in the second position of the electrodes do not contradict this supposition; they are obtained upon the portion of ventricle which is presumably least removed from the normal, and the results of excitation conform with the normal course of the excitatory variation which takes place in the stunned Frog's heart; it was the first instance which we observed of a regular diphasic variation indicative of the passage of the excitatory state from B to A or from A to B. The direction of the spontaneous variation in this case is such as to indicate that the negativity is most manifest at B, but that it is first manifested at A. It is probable that we have to do here with a weak first and an intensified second phase of a diphasic variation proceeding from A to B.

Experiment 11.—Spontaneous variations are such as to indicate an excitatory change (negativity) commencing at B and terminating at A. Excitation at A gave the reverse effect, *viz.*, a diphasic variation NS. We attempted to follow the variation by the rheotome in this case, but met with the difficulty that we were unable to obtain unfailing effects with short intervals between successive excitations, and that the prolongation of the experiment allowed of a too considerable decline of excitability for it to be possible to take observations with several positions of the rheotome. The necessity for allowing considerable intervals between test excitations was a noteworthy feature in all our experiments; the refractory period of excitation appears to be greatly prolonged in the excised Mammalian heart. For these reasons, and also because of the difficulty of knowing beforehand what intervals and what periods of closure to adopt for rheotome investigation, we did not pursue our experiments with this instrument.

TABLE I.—Abstract of Galvanometer Experiments—(continued).

| Experi- ment. | Date. | Animal. | P.M. | Spon- taneous beats. | Apex excitation. | Base excitation. | Demarca- tion. | Time P.M. of registered demarkation. |
|------------------|----------------|---------|--------|----------------------------|---------------------|---------------------|-------------------|--|
| 12 | 1886 Jan. 2 | Cat . . | 30' | S & SN | NS & N | SN & S | N ? | |
| " | " | " . . | Later | NS | NS | SN | | |
| 13 | Jan. 4 | Cat . . | 30' | "S & S | NS | S | | |
| 14 | Jan. 5 | Cat . . | 5'-30' | S | NS | S | | |
| " | " | " . . | 1 hr. | " | S | SN | | |
| 15 | Jan. 6 | Cat . . | " | S | NS | sS | | |
| 16 | Jan. 6 | Cat . . | " | S | NS | | | |
| 17 | Jan. 7 | Cat . . | " | | NS | S | N | |

Experiment 12.—The observations within an hour after excision were very uniform as regards excitatory effects, viz., diphasic variations according to the point of excitation; in the second half of the first hour we noticed that, whereas a stronger stimulus was still capable of giving typical diphasic variations, viz., NS by excitation at A, SN by excitation at B, a weaker stimulus gave only a single variation, viz., N by excitation at A, S by excitation at B. Presumably, the excitability was not far removed from uniformity, and with this agrees the fact that the two contacts were practically iso-electric. The spontaneous beats indicated the passage of an excitatory change from B to A.

Later, in the second half-hour, the excitatory effects continued uniform, viz., NS by excitation at A, SN by excitation at B; the noteworthy point during this period was the appearance of spontaneous variations NS; these were not properly spontaneous, but consequent upon excitation, each stimulus at A giving not merely one variation NS, but a series of two, three, or more such variations at intervals.

Experiment 13.—This agrees with the supposition that the apex is more injured than the base: we omitted to take the demarcation current.

Experiment 14.—The above remark applies to this experiment during the first half-hour.

Experiment 15.—Our results are compatible with the supposition that A is warmer than B, and that the excitatory variation at B appears twice.

Our attempt to reproduce the effect by warming B or cooling A failed.

Experiment 16.—We tried on this heart the effects of thermal injury, bringing a heated point near the apex; it gave a variation S, viz., apex positive; thermal injury near the base gave an N variation, i.e., base positive; these anomalous results we afterwards found to be due to thermoelectric currents.

Experiment 17.

Cat's heart.—Galvanometer observations begin 25' post mortem. Apex to N screw of galvanometer.

I. Electrodes on *left ventricle*. Demarcation current N.

| | | |
|--------------------------------------|-----|-----------------------|
| Apex excitation | NS | |
| Base " | S | |
| Apex " | NS | |
| Base " | S | followed by delirium. |
| Weak apex excitation | N | only. |
| Apex excitation | NS | |
| Right ventricle excitation | | no effect. |
| Base excitation | SNS | |
| Base " | SNS | |
| Apex " | NS | |
| Base " | SNS | |
| Weak base excitation | S | |
| Strong base " | SNS | |
| Apex excitation | NS | |
| Both auricles excited | | no effect. |

II. Electrodes on *right ventricle*

| | | |
|---------------------------|----|-----------------------|
| Apex excitation | N | |
| Base " | SN | followed by delirium. |
| Apex " | NS | |
| Base " | SN | |

TABLE I.—Abstract of Galvanometer Experiments—(continued).

| Experiment. | Date. | Animal. | P.M. | Spontaneous beats. | Apex excitation. | Base excitation. | Demarcation. | Time P.M. of registered demarcation |
|-------------|-----------------|----------|---------|--------------------|------------------|------------------|--------------|-------------------------------------|
| 18 | 1886 Jan. 15 | Cat . . | . | S | S | S | N | |
| 19 | Jan. 15 | Cat . . | .. | SN | NS & N | S | | |
| 20 | Jan. 13 | Dog . . | 2 hrs. | NS | N | S | N | 120' |
| 21 | Jan. 22 | Cat . . | 5'-15' | SN | NS | SN | S | 5' |
| 22 | Jan. 22 | Cat . . | 8' | SN | NS | SN | | |
| 23 | Jan. 27 | Dog . . | 10'-15' | .. | NS | S | N | 10' |
| 24 | Jan. 28 | Dog . . | 2' | N | N | SN | | |
| 25 | Jan. 30 | Dog . . | | N | N | SN | | |
| 26 | Feb. 5 | Cat . . | 25' | . | N | S | | |
| 27 | Feb. 9 | Sheep . | .. | . | NS | SN | N '012 D | 26' |
| 28 | Feb. 12 | Rabbit . | 25' | S | NS | S & SN | | |

Experiment 17 (continued).

Spontaneous beat NS

Base excitation SN

Base " S

1 hour post mortem excitatory effects uncertain.

Injury at base S

Injury at apex N

Change caused by apex injury less than that caused by base injury.

Experiment 19.—We noted that 15 mins. post mortem the spontaneous variation was SN, at 34 mins. post mortem it was N.*Experiment 21*—We cooled this heart by placing it in a vessel surrounded by melting ice; in the course of cooling we observed the following effects of excitation at A: NS, NSN, N, and nothing. The variation NSN is compatible with A cooler than B; the variation N is due to the excitatory process not reaching to B.*Experiment 23*—These effects are compatible with greater injury at apex than at base. We tried the effects of excitation with the electrodes on the two borders of the left ventricle, and obtained by excitation near N the variation NS; near S the variation SN.

On stimulating midway between N and S, we obtained no appreciable variation.

On exciting nearer to N, we obtained NS; on exciting nearer to S, we obtained SN.

Similar effects were obtained on the right ventricle.

Spontaneous effects were now observed N, N and S being on apex and base.

Apex excitation gave N.

Base excitation gave SN.

Later (56 mins post mortem) we observed spontaneous variation SN on the L.V.

59 mins post mortem we observed spontaneous variations on the R.V., N; on the L.V., S.

Experiment 24.—Tested laterally on the L.V., we obtained from excitation near A a variation NS, from excitation near B a variation SN.

With A and B at apex and base of R.V., we obtained, 2 hours and 20 mins. post mortem, a series of spontaneous variations SN.

Experiment 25.—We took the spontaneous variation leading off from the right and left borders of the L.V., left border to S electrode, right border to N electrode, and observed it to be SN; this variation indicates negativity, beginning on the left side, ending on the right side.

2½ hours post mortem, spontaneous beats NS were still apparent, the R.V. being led off in the usual manner from A and B.

Experiment 26.—Extreme susceptibility to injury; the slightest touch gave permanent deflections; the current of injury was '014 A.*Experiment 27.*—Tested laterally, A being on the right border and B on the left border of the L.V., we obtained, by excitation near A, NS; by excitation near B, SN; spontaneous variation, S.

On the R.V., laterally, we obtained similar effects by excitation near A and B respectively, viz, NS from excitation of A; SN from excitation of B. Spontaneous variation was NS, A being on the left border, B on the right.

TABLE I.—Abstract of Galvanometer Experiments—(continued).

| Experi- ment. | Date. | Animal. | P.M. | Spontaneous beats. | Apex excitation. | Base excitation. | Dema- rcation. | Time P.M. of registered dema-rcation. |
|------------------|-----------------|----------|------|-----------------------|---------------------|---------------------|-------------------|---|
| 37 | 1886 March 1 | Rabbit . | . | . | N | S | N | 0112 |
| 38 | March 2 | Rat . . | .. | . | N | S | N | 0006 |
| 39 | March 8 | Kitten . | | .. | N | S | S | |
| 40 | March 8 | Kitten . | | S | NS | SN | | |
| 41 | March 8 | Kitten . | . | NS | NS | S & SN | N | |
| 42 | March 15 | Dog . | | N | NS | S | S | |
| 43 | March 20 | Dog . | .. | .. | NS | S | S | |
| 44 | March 26 | Rabbit | .. | S | NS | S | | |
| 45 | March 27 | Cat. | | | | | | |
| 46 | June 6 . | Dog . . | | S | NS | SN | N | |

Experiment 44 furnished us with good data for the comparison of galvanometric with electrometric indications. The electromotive change accompanying the spontaneous beats was galvanometrically S, electrometrically NS; the latter we take to be the correct expression of the actual change. It proves beyond a doubt that the excitatory change (negativity) manifested itself at apex and then at base. When we shifted the electrode B to the auricle, which was giving two, three, or four beats to one ventricular beat, the electrometer showed a small South variation, corresponding to each beat of the auricle alone, followed by a larger double variation North-South with each ventricular beat. On moving electrode B to its original position at the base of the ventricle, the auricular beats ceased to affect the electrometer. The result obtained above we regard as most significant: it shows auricular negativity, followed by a biphasic ventricular negativity, at apex first, then at base; and it proves beyond doubt that the transmission of the excitatory state takes place, otherwise than in the Frog's heart, probably by nervous channels. If the excitatory state had passed from auricle to adjacent part of the ventricle, and thence to the apex by muscular continuity, its E.M. expression would necessarily have been a diphasic variation South-North.

Experiment 45.—The spontaneous variation immediately after excision was *South*, i.e., base negative; the excited variation was North-South with excitation of apex, South-North with excitation of base.

TABLE K.—Abstract of Electrometer Observations.

| No of experiment. | Animal. | Spontaneous. | Apex excitation. | Base excitation. | Remarks. |
|-------------------|----------|----------------------|------------------|------------------|--|
| 42 | Dog . . | SN | NS | S | |
| 44 | Rabbit . | NS | NS | SN | |
| 45 | Cat . . | S | NS | SN | |
| 46 | Kitten . | N and NS | | | |
| 47 | Rabbit . | N | . . . | | <i>Vide</i> Photo. 3. |
| 48 | Rabbit . | N | | | |
| 49 | Cat . . | NS | | | |
| 50 | Puppy . | NS | NS | S | |
| " | " . . | | | | Lateral excitation. Near N electrode NS. " S " SN. |
| 51 | Dog . . | S | | | |
| 52 | Puppy . | sN | | | |
| " | " . . | sN | | | |
| 53 | Puppy . | NS and N | . . . | | <i>Vide</i> Photo. 7. |
| 54 | Puppy . | SN | | | |
| 55 | Puppy . | N | N | | |
| 56 | Rabbit . | . . . | NS | | |
| 57 | Puppy . | N | | | <i>Vide</i> Photo. 6a. |
| " | " . . | NS | | | <i>Vide</i> Photos. 6β and 6γ. |
| 58 | Dog . . | . . . | N | SN | |
| 59 | Puppy . | NS | NS | | <i>Vide</i> Photo. 5. |
| 60 | Cat . . | S | . . . | | <i>Vide</i> Photo. 4. |
| " | " . . | NS | NS | S | |
| 61 | Cat . . | S and SN | N and NsN | SN | |
| 62 | Rabbit . | N and NS | N and NS | SN | |
| 63 | Cat . . | SN | NS | S | |
| 64 | Cat . . | N and NS alternately | N and NsN | SN | Lateral excitation S on R.V. Ss—SN. N on L.V. Ns—NS. |

XI. *Studies on some New Micro-organisms obtained from Air.*

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[PLATES 17–20.]

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In some papers on the micro-organisms present in air, previously communicated to the Royal Society by one of us,* the relative abundance of microbes in the air of different places has been called attention to and the methods of experiment fully described. As these investigations were carried out with the aid of solid nourishing media, we were able to obtain a collection of pure cultivations of a number of micro-organisms derived directly from the air. It appeared to us, therefore, desirable to utilise the opportunity which these experiments furnished for minutely characterising some of the principal forms which are thus obtainable from the atmosphere. There are many reasons which render it of importance that the task in question should be undertaken. Thus, in the methods of cultivation employed by bacteriologists, the experimenter may at any moment be brought face to face with organisms from the air which have accidentally contaminated his cultivations, and it is obvious, therefore, that an intimate acquaintance with the various forms which may thus invade culture-media must be both of interest and importance to all practically engaged in experiments on micro-organisms.

It is not unnatural that the brilliant discoveries in connection with the etiology of infectious diseases should have absorbed the lion's share of the attention of investigators in the field of bacteriology, and that the non-pathogenic organisms should have come to be regarded as comparatively uninteresting by the side of their more formidable brethren. It must, however, be remembered that the functions of the non-pathogenic organisms in the economy of nature are as yet but very imperfectly understood, and that as far as these functions have been investigated they do not yield in point of importance to those of the most virulent pathogenic forms.

Thus the conversion of sugar into alcohol, the decomposition of nitrogenous organic matter with elimination of ammonia, the oxidation of ammonia to nitrous and nitric acids, besides many other natural transformations which are effected through the agency of such micro-organisms, are certainly not second in importance to the results, terrible as they often are, achieved by the pathogenic forms. The organisms producing the above-mentioned changes are known to be present in the air, and there can be little doubt that the numerous other aerial varieties will in the future be found to discharge important duties in the laboratory of nature.

The exactness with which bacteriological research can now be carried out, thanks to the beautiful methods of cultivation which have been developed during the past six years, renders it imperative that all future investigations on the chemical and physiological action of micro-organisms should be made with specific organisms and not with mixtures, as has so often hitherto been the case. On this account the

* "The Distribution of Micro-organisms in Air," 'Roy. Soc. Proc.' vol. 40, 1886, p. 509.

"A New Method for the Quantitative Estimation of the Micro-organisms present in the Atmosphere," *ibid.*, vol. 41, 1887, p. 443; 'Phil. Trans.' 1887, B, p. 113.

"Further Experiments on the Distribution of Micro-organisms in Air (by Hesse's Method)," 'Roy. Soc. Proc.' vol. 42, 1887, p. 267.

first step in investigations of this kind must consist in such careful delineation of the characteristics of specific organisms that their identification may be readily accomplished. In this way it may with confidence be anticipated that the particular chemical and physiological properties of each specific organism will in the future be elaborated, as has been done in a few cases already. It has been especially with this object in view that we have undertaken the task of collecting from the air a number of different varieties of micro-organisms, and, after isolating and obtaining them in a pure state, to carefully delineate the characteristic appearances which they present, both under the microscope and when grown in various cultivating media.

Methods of Study and Examination.

The organisms which we have made the subject of special study were obtained in the examination of the air of various places by means of HESSE's tubes, and by the exposure of dishes filled with gelatine-peptone in the manner already described. In both cases the aerial organisms are deposited on the surface of the solid gelatine, and, by incubation for several days, each organism thus deposited gives rise to a colony frequently possessing a characteristic appearance. If these colonies are not too closely crowded together on the surface of the gelatine, it is easy to transfer a small portion of a single colony to a culture-tube without any admixture from adjacent colonies. This transference is most conveniently effected by means of a sterilised platinum-needle. As the colonies obtained in the HESSE's tubes or on the gelatine dishes are, as already pointed out by one of us, invariably pure, the cultivation which is obtained by inoculating the needle into a sterile culture-tube is also pure if the operation of transference is performed with care and in an atmosphere reasonably free from dust. Thus in the hundreds of cultivations which this investigation has entailed we have scarcely had a single instance of a culture being vitiated through contamination from the air during the process of transference and inoculation.

Gelatine tube-cultivations.—The colonies originally obtained from the air were invariably inoculated, in the first instance, into test tubes, one-third filled with solid sterile gelatine-peptone (for preparation of which see below), and plugged with cotton wool in the ordinary way. The appearances in these gelatine tubes have been carefully watched, described, and in many cases drawn. These appearances are, as is well known, of great importance in serving to characterise specific organisms, and frequently serve to discriminate between organisms of similar and almost identical microscopic appearance.

Composition of Cultivating Media employed:—

Gelatine.—100 grms. gelatine; 10 grms. peptone (dry); common salt, 1 grm.; lean beef, 1 lb; water 1 litre.

Agar-agar.—10 grms. agar-agar instead of the gelatine; otherwise identical.

Broth.—Similar to the above, only omitting the gelatine or agar-agar.

In making these inoculations, it has been our practice not only to pierce the needle to a distance of an inch or more into the depth, but also to streak it along the surface of the gelatine, as in this manner two characteristic growths are obtained, the one along the track of the needle beneath, and the other upon the surface of the gelatine.

In many instances the growth of the organisms is accomplished by liquefaction of the gelatine, and the manner in which this liquefaction takes place is often very characteristic, both as to the rapidity with which the change occurs and as to the form of the liquefied portion. The differences observed in respect of this phenomenon will be duly noticed in treating of the individual varieties of organisms.

Agar-agar tube-cultivation.—The organisms were in every case also inoculated into similar test-tubes containing agar-agar mixture (for preparation see note, p. 259), and the appearances presented by their growth in this medium have been studied, described, and, where particularly characteristic, drawn.

The agar-agar cultivations frequently serve to establish differences between organisms which, owing to their liquefaction of gelatine, do not furnish characteristic growths in that medium, for the agar-agar is not liquefied by any organisms, and thus surface-growths can be obtained in the case of those which in gelatine produce only liquefaction. On the other hand, there are many cases in which the cultivations on agar-agar are but little characteristic, whilst in gelatine the same organisms present important marks of distinction.

Broth-cultivations.—The organisms were also in every case inoculated into test-tubes containing sterile broth-peptone (for preparation see note, p. 259), and the appearances presented by their growth in this medium have also been carefully observed and described. The appearance of the broth cultivations is generally very much less characteristic than those in gelatine or agar-agar, the principal differences observable being in respect of the formation or non-formation of a pellicle on the surface of the liquid.

There is, however, a special reason for carrying out cultivations in broth, and that is that the form of the individual organisms is generally more uniform and natural in a fluid than in a solid culture-medium, for in the latter the forms are occasionally distorted and rendered abnormal by the pressure to which they may be exposed.

Gelatine plate-cultivations.—Not unfrequently the most striking appearances are presented by micro-organisms when growing in colonies on gelatine-plates. The colonies are often highly characteristic even to the naked eye, and they generally become far more so when examined by transmitted light with a low magnifying power (about 100 diameters); these appearances we have in all cases carefully described and drawn.

The plate cultivations are prepared by inoculating with a needle from a tube cultivation into a sterile test-tube containing melted gelatine, thoroughly mixing the latter by agitation, then inoculating with a needle from this into a second test-tube, which is again mixed by agitation. The gelatine in this second test-tube is then

poured out upon a sterile glass-plate and allowed to congeal. Sometimes even a further attenuation is prepared by inoculating from the second test-tube into a third, which is also poured out upon a plate.

In this manner one or other or both of the plates is almost sure to yield colonies sufficiently separated from each other to prevent interference and to enable further inoculations to be made from a single colony.

By means of this method of plate-cultivation we have also controlled the purity of all our other cultivations. For, on submitting the contents of any culture-tube to plate-cultivation in this manner, any impurity in the original culture will become apparent through the production of dissimilar colonies on the plate.

The colonies are also of great importance for the preparation of cover-glasses (see later below) for microscopic examination with a high power (1,000 diameters), as all the forms obtained from a single colony may with certainty be known to belong to a single organism.

We must point out the great necessity of fully describing the appearance of all organisms when growing in colonies, as this forms one of the most important aids in the discrimination and identification of organisms.

Microscopic examination.—In addition to the macroscopic observations already referred to, we have examined, measured, described, and drawn all the various organisms as they appear when viewed under a high power of the microscope. For this purpose we have, in general, employed a $\frac{1}{1}$ oil-immersion (LEITZ) objective, with a No. 3 eye-piece, thus obtaining a magnifying power of nearly 1,000 diameters, whilst for the examination of micrococci we have also employed LEITZ'S $\frac{1}{30}$ objective, with the same eye-piece, thus obtaining a magnifying power of about 1,500 diameters.

The organisms have, in general, been prepared for examination according to the KOCH-LÖFFLER* method. Thus a small quantity of a cultivation is transferred, by means of a platinum needle, to a clean cover-glass, a small drop of sterilised distilled water is added, and the mixture is spread as thinly as possible over the glass with the aid of the platinum needle. A second cover-glass is now laid upon the first and then drawn off, each glass being now provided with a thin film of diluted cultivation. The two cover-glasses are laid down with their wet faces uppermost and allowed to become quite dry. One of the cover-glasses is then held by one corner with a pair of forceps and slowly passed three times through the flame of a Bunsen burner or spirit-lamp, the face bearing the film being held upwards. The dried and ignited specimen can then be preserved indefinitely before staining for examination.

The specimen is stained by running a few drops of a diluted alcoholic solution of an aniline dye (gentian-violet, magenta, methylene blue, &c.) with a pipette upon the cover-glass, which is held with forceps by one corner and moved about so as to cause the colouring matter to flow evenly over every part of the film. The dye is allowed to remain on the glass for about one minute, the exact time being varied according to

* 'Die Methoden der Bakterien-Forschung,' HUEPPE, Wiesbaden, 1885, p. 42.

the relative facility with which the organism has by experience been found to take the colouring matter; it is then washed off with distilled water, and, if intended for immediate examination, laid with the film-surface downwards upon a clean glass slip. The excess of water is then carefully removed with blotting-paper, and the preparation is ready for examination. If the preparation is intended for permanent preservation, it is, after washing off the dye, allowed to dry, and then mounted with a drop of Canada balsam.

Drop-cultivations.—We have also studied the motility and progressive growth of the various organisms in “drop-cultivations.” The drop-cultivations are prepared as follows:—

A cover-glass is sterilised by holding it with a pair of forceps and passing it several times through a Bunsen-flame or spirit-lamp. A glass slip having a round excavation on one surface, and capable of being completely covered by the cover-glass, is similarly sterilised, and both cover-glass and slip are allowed to cool under a glass shade. When cool, a small drop of sterile broth is placed by means of a sterile looped platinum needle in the centre of one surface of the cover-glass, and immediately after a very minute quantity (the smaller, the better) of a cultivation of the organism under examination is introduced by means of a sterile platinum needle into the drop of broth, and the cover-glass bearing the latter is then carefully placed so that the drop upon its under-surface projects into the centre of the excavation. The periphery of the excavation has been previously coated with a thin layer of vaseline, so that when the cover-glass is placed in position as described the vaseline produces an air-tight seal to the small circular cell which is thus formed.

If the above manipulations are performed with due care, a perfectly pure cultivation is obtained, and its progress can be observed under the highest powers of the microscope.

By the aid of the above-described methods we have endeavoured to accurately characterise a number of micro-organisms which we have obtained from air. A few of these, such as the *Micrococcus prodigiosus*, the *Bacillus subtilis*, the yellow and orange *Sarcina*, have been more or less closely described by other observers, but the remainder are entirely new, or, if previously observed, they have not to our knowledge, after careful reference to the most recent literature, been so described as to render them capable of identification. Under these circumstances we have felt it necessary to provisionally give names to all those forms which have not been hitherto described. The names which we have selected for this purpose are generally of such a nature as to indicate some striking peculiarity which the organisms present either in their cultivations or when viewed under the microscope. By adopting this plan, we believe that the description of, and reference to, the organisms which we have had under observation will be facilitated more than if we had only designated them by numbers or other symbols.

In our terminology we have, following the practice of many recent writers, abandoned the term *bacterium*, distinguishing those forms which are distinctly elongated as *bacilli*, and those which are spherical, or approximately so, as *micrococci*.

I.—MICROCOCCL.

The following different varieties of micrococci have been found and examined by us :—

1. *Micrococcus carnicolor*.
2. *M. albus*.
3. *Streptococcus liquefaciens*.
4. *Sarcina lutea*.
5. *S. aurantiaca*.
6. *S. liquefaciens*.
7. *M. gigas*.
8. *M. chryseus*.
9. *M. rosaceus*.
10. *M. candicans*.

1. MICROCOCCUS CARNICOLOR.

Occurrence.—This micrococcus was obtained as a pinkish surface-expansion on a gelatine-dish exposed to the air of the Close at the base of Norwich Cathedral, 26th April, 1886.

Microscopic appearance.—Under the high power ($\times 1,000$) it is seen to consist of almost round cocci varying in size from 0.5μ to about 1.5μ . The larger forms almost invariably exhibit a division (see Plate 17, fig. 2, No. 2c). Otherwise the cocci present no definite arrangement.

When viewed in drop-cultivations, they exhibit the usual vibratory motion of micrococci.

Appearance in Cultivations.

Gelatine.—In tube after four days (August 6—10, 1886) the needle-track in the depth exhibits but very slight growth; over the surface, however, there is a pink expansion extending laterally on either side of the streak of the needle. At a later period the needle-track beneath the surface becomes beaded, and the colour of the surface-growth is seen to be of a lighter tint in the centre than round the periphery. (See Plate 17, No. 2b.)

In very old cultivations the gelatine becomes slightly liquefied.

Agar-agar.—Grows rapidly, producing a smooth flesh-coloured surface-expansion having a glazed appearance. (See Plate 17, No. 2a.)

Broth.—After nine days (August 7—16, 1886) the liquid is clear, free from a pellicle, and has a pinkish deposit at the bottom.

Appearance on plate-cultivation.—The colonies are seen to the naked eye to be of a faint pink colour. Under a low power ($\times 100$) they appear as almost perfectly circular smooth-edged colonies, the interior of which is exceedingly finely granular in its nature. They are brown in colour, the shade becoming deeper as the colonies develop further. (See Plate 17, No. 2*d*.) When the colonies reach the surface of the gelatine they form a very thin and round—almost colourless—expansion, which subsequently acquires the characteristic pink tint. Under a low power this expansion is seen to be finely granular, with an almost perfectly smooth edge. (See Plate 17, No. 2*e*.) Tubes were inoculated both from the surface-colonies and from those in the depth of the gelatine, and from each the same characteristic growth was obtained.

This organism has many points of similarity with the *Micrococcus rosaceus*, from which it is chiefly distinguishable (1) by its more rapid growth, (2) by the fainter colour of the pigment, and (3) by the different appearance of its colonies. (See page 269.)

2. MICROCOCCUS ALBUS.

Occurrence.—We have obtained this as a white surface-expansion on a gelatine-dish from the same place as the *Micrococcus carnicolor* (p. 268).

Microscopic appearance.—Viewed under a high power ($\times 1,000$), this is seen to consist of cocci varying in size from 0.8μ to 1.5μ , the larger ones presenting a division (see Plate 17, fig. 5, No. 5*b*); they have no characteristic arrangement.

Appearance in Cultivations.

Gelatine.—In tube after four days (August 6—10, 1886) the needle-track below the surface shows a faint saw-like growth, whilst on the surface there is a narrow, white, shining expansion spreading on either side of the needle-streak. The edge is lobular (see Plate 17, No. 5*a*) and smooth, not serrated. No liquefaction of the gelatine takes place.

Agar-agar.—It appears as a faintly white, almost colourless surface-expansion, with a smooth, but lobular, edge.

Broth.—After nine days (August 7—16, 1886) the liquid is very slightly turbid, free from pellicle, with a yellowish-white deposit at the bottom.

Appearance on plate-cultivation.—The colonies are visible to the naked eye as small milk-white discs. Under a low power ($\times 100$) they are seen to be circular, sharp-edged, and finely granular in nature. In colour they exhibit varying shades of brown, according to the degree of their development. (See Plate 17, No. 5*c*.)

3. STREPTOCOCCUS LIQUEFACIENS.

Occurrence.—This was obtained from air as a yellow surface-expansion on a gelatine-dish.

Microscopic appearance.—Under a high power ($\times 1,000$) this is seen to be a small micrococcus, varying in size from 0.5μ to 0.8μ , the dimensions being thus fairly uniform. The cocci are arranged in short chains, as seen in Plate 18, fig. 3, No. 3a.

Appearance in Cultivations.

Gelatine.—After four days (August 6—10, 1886) the needle-track below the surface is very faint; at the top there is a slight depression, with a light lemon-yellow deposit, slight liquefaction of the gelatine having there taken place. As the cultivation becomes older, the liquefaction slowly proceeds, the needle-track in the still solid gelatine remaining very faint.

Agar-agar.—Forms an almost colourless shining growth extending even after a month, but slightly to left and right of needle-streak.

Broth.—After nine days (August 7—16, 1886) the liquid is clear, free from pellicle, and has a dirty yellowish-white deposit.

Appearance on plate-cultivation.—To the naked eye the colonies appear as yellowish pin-heads on the surface, each being surrounded by a slight depression. Under a low power ($\times 100$) the smaller colonies appear irregularly circular (see Plate 18, No. 3b), the edge is smooth, and the interior is more or less granular.

In gelatine the growth of this organism presents points of similarity to that of *Sarcina lutea*, from which, however, it is most sharply distinguishable, not only by its appearance under the microscope, but also by its growth in agar-agar.

4. *SARCINA LUTEA.*

Occurrence.—We have not ourselves found this organism in the air, but a cultivation of the same was brought by one of us from Dr. KOCH's laboratory at the Hygienic Institute of Berlin.

It has already been partially described by KLEIN ('Micro-organisms and Disease,' 1885, p. 48), EISENBERG ('Bakteriologische Diagnostik,' 1886), CROOKSHANK ('Introduction to Practical Bacteriology,' 1886, p. 120), FLUGGE ('Die Mikro-Organismen,' 1886, p. 179).

Microscopic appearance.—Under a high power ($\times 1,000$) there are seen large cocci, mostly grouped together in cubical packets of four or more. The individual cells vary in diameter from 1.5μ to 2.5μ , and are best seen when lightly stained with methylene-blue. The staining is very liable to be too intense and so prevent the grouping being recognisable, owing to their lying in heaps, the division of the cells taking place both vertically and horizontally. (See Plate 18, fig. 6, No. 6b.)

The appearance in drop-cultures is particularly characteristic, the arrangement in cubical packets being most beautifully shown. It is, of course, non-motile.

Appearance in Cultivations.

Gelatine.—In the tube it grows slowly, forming numerous minute yellow centres in the track of the needle, whilst on the surface it produces a shining lemon-yellow

expansion consisting of small hump-like protuberances. In nine days (August 6—15, 1886) the surface-growth was still very restricted, but had formed a depression filled with lemon-yellow semi-liquid matter. Even after eighteen days (August 6—24) there was but little change in the needle-track, but the surface-depression, which was considerable (see Plate 18, No. 6a), was filled with liquid, at the bottom of which was a lemon-yellow deposit.

This organism thus causes a very slow liquefaction of the gelatine, and produces a very decided lemon-yellow pigment.

Agar-agar.—Forms a thick chrome-yellow moist mass extending over the surface.

Broth.—After nine days (August 7—16, 1886) the liquid is clear and free from pellicle; there is a lemon-yellow deposit at the bottom.

Appearance on plate-cultivation.—The colonies are visible to the naked eye as small yellow centres which, under a low power ($\times 100$), appear of irregular shape, finely granular near the periphery, the edge being nearly smooth (see Plate 18, No. 6c). The centre of the colony has a dark greyish-green colour. No liquefaction was observed during the first six days.

5. *SARCINA AURANTIACA*.

Origin.—This organism is also occasionally found in the air, although we have not ourselves met with it there. A cultivation was brought by one of us from Dr. KOCH's laboratory in Berlin. The only references to this organism which we have been able to find are in EISENBERG's 'Bakteriologische Diagnostik,' which contains a very brief description of its appearance, and its existence is mentioned by FLÜGGE ('Die Mikro-Organismen') without, however, any description being appended.

Microscopic appearance.—Under the high power ($\times 1,000$ or $\times 1,500$) there are seen packets of cocci which are much smaller than those of *Sarcina lutea*. The complete packet of four cocci measures only about 1.7μ across. (See Plate 18, fig. 4, No. 4b.)

Appearance in Cultivations.

Gelatine.—In the tube, after four days (August 6—10, 1886), liquefaction has taken place along the path of the needle, producing a funnel-shaped canal which is filled with clear liquid, at the bottom of which is a flocculent orange deposit.

The Plate 18 (No. 4a) exhibits the condition of the cultivation after seven days' growth (August 6—13); the liquefaction even then has not extended across the tube, and the lower extremity of the needle-path is still comparatively undeveloped.

Agar-agar.—Forms an abundant and moist surface-growth of a strong orange colour. The growth is for the most part continuous, but numerous little heaps are distributed over the remainder of the surface.

Broth.—After nine days (August 7—16, 1886) the liquid is turbid at the surface,

but clear below, with a dirty white deposit at the bottom. After eighteen days the deposit has become of an orange colour.

Appearance on plate-cultivation.—To the naked eye the colonies are visible on the fifth day (October 13—18, 1886) as small, round, yellow colonies, each of which exhibits a circular surface-depression of varying size. On examination with a low power ($\times 100$), the colonies are seen to be circular and granular, with a slightly denticulated edge, which in the less developed colonies is not so marked. (See Plate 18, No. 4c.)

6. *SARCINA LIQUEFACIENS*.

Occurrence.—We have found this organism in the air collected on the roof of the Science Schools, South Kensington Museum. It was particularly abundant on the 8th July, 1886, when it was found producing small granular liquefying colonies on the surface of gelatine-dishes which had been exposed there.

Microscopic appearance.—Under a high power ($\times 1,000$ or $1,500$) it much resembles *Sarcina lutea*, the cocci, which are about 1.5μ in diameter, being arranged in packets of four and upwards, a very large number sometimes remaining aggregated together. (See Plate 18, fig. 5, No. 5b.)

Appearance in Cultivations.

Gelatine.—After four days (August 6—10, 1886) the needle-track below is composed of small isolated whitish centres, whilst above there is a large depression with an air-space and cloudy liquid contents, at the bottom of which there is a greyish-white deposit. The liquefaction has not extended across the tube. On the ninth day (August 6—15) the liquefaction has extended across the tube to depth of about half-an-inch (see Plate 18, No. 5a), the liquid portion being very turbid, with a yellowish-white deposit resting upon the surface of the still solid gelatine below. The lower portion of the needle-track exhibits no material alteration. Subsequently the liquid portion becomes quite clear.

Agar-agar.—The growth is very rapid, producing an almost colourless (very faintly green) expansion, very much resembling that of *Sarcina aurantiaca*, excepting as regards the colour.

Broth.—After nine days (August 7—16, 1886) the liquid is clear, free from pellicle, and with a dirty-white deposit at the bottom, which subsequently becomes of an orange colour.

Appearance on plate-cultivation.—To the naked eye the colonies appear almost colourless (very faintly green). They had not caused liquefaction of the gelatine on the fifth day (October 13—18, 1886); a day later (October 19) they formed a surface-depression like *Sarcina aurantiaca*. Under a low power ($\times 100$) the colonies appear (see Plate 18, No. 5c) as highly irregular in contour, with a denticulated and lobular edge and granular contents.

Distinctive Differences existing between the three Forms of Sarcina described.

Under the microscope the *Sarcina aurantiaca* is sharply distinguished from the other two by the smaller size of its cells, whilst it presents a still more striking contrast to the other two in the colour of the pigment which it produces when cultivated in gelatine or on agar-agar. From *Sarcina lutea* the other two are also distinguished by the far more rapid liquefaction of the gelatine which they produce. The property which the almost colourless *Sarcina* has of liquefying right across the tube presents a marked point of distinction from *Sarcina aurantiaca*, in which the liquefaction takes place in the form of a bag.

7. MICROCOCCUS GIGAS.

Occurrence.—This was found by us in the air of a cow-shed, forming a large white expansion on a gelatine dish exposed there.

Microscopic appearance.—Under a high power ($\times 1,000$ or $1,500$) this is seen to be a large micrococcus, sometimes as much as 1.7μ in diameter; the cocci are frequently adherent in pairs. (See Plate 17, fig. 3, No. 3a.)

Appearance in Cultivations.

Gelatine.—It liquefies the gelatine slowly, rendering it turbid.

Agar-agar.—It forms a yellowish-white smooth surface-growth, extending in lobules to right and left of the needle-streak. Later on, the colour becomes cream-yellow, and the lobules, which are numerous, remain small. There is a considerable granular growth in the track of the needle beneath the surface.

Broth.—After six days (September 7—13, 1886) the liquid is clear, free from pellicle, and has a whitish deposit at the bottom.

Appearance on plate-cultivation.—After four days the colonies appear on the surface as pin-heads of a faint cream colour, each causing a depression in the gelatine. Under a low power ($\times 100$) the colonies are seen to be circular in shape, with a slightly irregular edge, and the contents, which are cloudy at the centre, become distinctly granular towards the edge. (See Plate 17, No. 3b.)

8. MICROCOCCUS CHRYSEUS.

Occurrence.—This was found by us in the air collected on the roof of the Science Schools, South Kensington Museum.

Microscopic appearance.—Under the high power ($\times 1,000$ or $1,500$) it appears as a micrococcus of variable size, up to 1μ in diameter; the largest cells exhibit a division. The cocci are not arranged in any definite manner. (See Plate 19, fig. 3, No. 3a.)

Appearance in Cultivations.

Gelatine.—After four days (August 6—10, 1886) there is a slight surface depression filled with semi-liquid cream-coloured matter; even after sixteen days the semi-liquefaction has but very slightly increased.

Agar-agar. It forms a surface shining growth of light-orange colour.

Broth.—After nine days (August 7—16, 1886) the liquid is clear, free from pellicle, and has a dirty-white deposit at the bottom.

Appearance on plate-cultivation.—After four days the surface-colonies are visible to the naked eye as pin-heads of yellowish colour. Under a low power ($\times 100$) they are seen to be generally round (see Plate 19, No. 3b), the more developed colonies showing a finely granular edge, whilst the less developed have a smooth edge.

NOTE.—The dark semi-circular edge seen in the figure indicates the depression produced by the colony on the surface of the gelatine.

9. *MICROCOCCTUS ROSACEUS.*

Occurrence.—We have frequently met with this organism in the course of our experiments on air; we have also compared it with a cultivation which was brought by one of us from Dr. KOCH's laboratory in Berlin. On gelatine plates or dishes which have been exposed to the air, it produces small, smooth, shining, bright pink expansions.

Microscopic appearance.—Under a high power ($\times 1,000$ or $1,500$) the cocci are seen to be very variable in size, the largest being as much as 2.5μ in diameter; the larger forms exhibit a well-marked division. (See Plate 17, fig. 4, No. 4a.)

Appearance in Cultivations.

Gelatine.—It forms a shining, smooth, pink expansion on the surface, whilst the needle-track below remains almost undeveloped. As the cultivation becomes older, the margin assumes a radiated appearance. Still older cultivations frequently exhibit slight liquefaction.

Agar-agar.—Forms a smooth, bright-pink surface-expansion devoid of any further characteristics.

Broth.—After nine days (Aug. 7—16, 1886) the liquid is clear, free from pellicle, and exhibits a pink deposit.

Appearance on plate-cultivation.—To the naked eye the more developed colonies appear as pin-heads on the surface, and are bright-pink in colour. Under a low power ($\times 100$) they are seen to be of a distinctly reddish tint, the edge being irregular, but smooth; but as the colonies approach the surface the irregularity diminishes. (See Plate 17, No. 4b.)

10. MICROCOCCUS CANDICANS.

Occurrence.—This was found in the air collected on the roof of the Science Schools, South Kensington Museum. We believe this organism to be identical with that described by FLÜGGE, *loc. cit.*, p. 173, but he does not mention that it liquefies gelatine.

Microscopic appearance.—Under a high power ($\times 1,000$ or $1,500$) the cocci are seen to be variable in size, the larger ones exhibiting a division and reaching 1μ in diameter; they are devoid of any definite arrangement. Plate 17, fig. 1, No. 1c, represents the appearance of the cocci taken from an agar-cultivation and viewed with a magnifying power of 700; in No. 1d they are taken from a gelatine-cultivation and are magnified 1,000 times.

Appearance in Cultivations.

Gelatine.—After four days there is a surface-depression containing an intensely white and opaque mass. As the cultivation becomes older, liquefaction slowly proceeds downwards, the liquid formed being highly glutinous and turbid. (See Plate 17, No. 1b.) The mode of liquefaction in the case of this organism is very dependent upon temperature; thus in warm weather, or if the temperature is maintained at about 22°C ., the liquefaction takes place in a long funnel, as seen in the Plate, whilst at a low temperature the liquefaction is mostly confined to the surface.

Agar-agar.—Already in the course of three days there is a strong growth forming a smooth and dazzling white mass upon the surface. The brilliancy of the white mass, which resembles a moist patch of Chinese white, is especially characteristic. (See Plate 17, No. 1a.)

Broth.—After nine days (August 7—16, 1886) the liquid is pervaded with a fine turbidity; there is no pellicle, but a white deposit is found on the bottom.

Appearance on plate-cultivation.—The colonies are milk-white and, under a low power ($\times 100$), they are seen to have a smooth edge, the interior being granular; and, whilst the older colonies are somewhat irregular in shape, the less developed ones are nearly circular. (See Plate 17, No. 1e.)

II.—BACILLI.

1. *Bacillus aureus.*
2. „ *aureus.*
3. „ *citreus.*
4. „ *plicatus.*
5. „ *chlorinus.*
6. „ *polymorphus.*
7. „ *profusus.*
8. „ *pestifer.*

9. *Bacillus lævis*.
10. „ *cereus*.
11. „ *subtilis*.
12. „ (*Micrococcus*) *prodigiosus*.

The above is a list of the various forms of bacilli which have been found by us in air, and which, with the exception of the two last, have not, as far as we are aware, been previously described. We have again ventured to designate these new forms by names which are indicative of some striking characteristic which they possess. Thus in the case of Nos. 1, 2, 3, and 5, the pigments which are produced on cultivation being very marked, the names have been selected with regard to this property. In the case of No. 4, again, the peculiar appearance of the cultivations is suggested in the name; whilst in No. 7 the microscopic appearance, and in No. 8 the strong and highly disagreeable smell possessed by its cultivations, are indicated by the names assigned to them.

1. *BACILLUS AURESCENS*.

Occurrence.—This was met with by us as a yellow growth on a gelatine-dish which had been exposed to the air of a railway-carriage.

Microscopic appearance.—Under a high power ($\times 1,000$ or $1,500$) this is seen to be a short bacillus occurring singly, in pairs, and in threads of three and four. The individual bacilli are from three to five times as long as broad, with rounded ends. Their length varies from 1.5μ to 3.5μ . In the threads the divisions are not always distinctly visible, and it has then the appearance of a long slender bacillus. In Plate 19, fig. 4, No. 4b, the appearance of the bacilli when grown in broth is represented, the magnifying power being 1,000. In No. 4c the bacilli are taken from a gelatine-cultivation, and are only magnified about 600 times.

Viewed in drop-cultivations, they exhibit vigorous vibratory and rotatory motion, but no movement of translation was observed.

Appearance in Cultivations.

Gelatine.—The growth is very faint in the track of the needle below, but on the surface it forms a light orange-coloured, *dry*, and much crumpled expansion, which does not cause liquefaction of the gelatine even in very old cultivations. The appearance is very characteristic.

Agar-agar.—Forms a *dry* light-orange surface-growth, much crumpled, with an irregular edge, which is of lighter colour than the central portion. (See Plate 19, No. 4a.)

Broth.—After six days (August 7—13, 1886) the liquid is clear, but there is a

plentiful deposit of cream-yellow matter, and the surface is covered with a delicate dirty-white pellicle, which subsequently becomes faintly cream in colour.

Appearance on plate-cultivation.—To the naked eye the colonies are visible as small pin-heads of a faint orange colour. Under a low power ($\times 100$) they are seen to be not perfectly circular, finely granular inside, and with a very slightly jagged edge. (See Plate 19, No. 4d.)

2. BACILLUS AUREUS.

Occurrence.—This was also found forming an orange-coloured pin-head on a gelatine-plate, which had been exposed in the same place as the last.

Microscopic appearance.—With a high power ($\times 1,000$ or $1,500$) this is seen as a bacillus forming fine graceful threads (see Plate 19, fig. 5, No. 5b), which are considerably longer than those formed by *Bacillus ourescens*. In drop-cultivations they exhibit vibratory motion only.

Appearance in Cultivations.

Gelatine.—There is but little growth in the path of the needle below, but on the surface it forms a dry crumpled expansion, which is of a much deeper orange colour than *B. aureus*. In old cultivations it causes slight liquefaction of the gelatine.

Agar-agar.—Forms an orange growth, which is less crumpled and less dry in appearance, but deeper in colour than that of *B. aureus*. (See Plate 19, No. 5a.) The cultivations, from which the drawings of these two bacilli were made, were started on the same day, and, although all the conditions were precisely similar, the difference between the two growths was very marked.

Broth.—After six days (Aug. 7—13, 1886) it resembles *B. aureus*, but the deposit and pellicle were deeper in colour.

Appearance on plate-cultivation.—The colonies differ but little from those of *B. aureus*, forming pin-heads on the surface, which are, however, of a deeper orange colour, and are more rapid in their growth. See Plate 19, No. 5c.

3. BACILLUS CITREUS.

Occurrence.—This was found producing a yellow pigment on the surface of a gelatine-dish which had been exposed to the air in Hyde Park.

Microscopic appearance.—Under a high power this is seen to be a short fat bacillus about one-and-a-half to twice as long as broad. It frequently exhibits a median transverse division, which can, however, be only well seen with a very high magnifying power ($\frac{1}{10}$ oil-immersion), $1,500$ times. Sometimes the bacilli hang together in chains of three and four. The average length of a pair is about 3.4μ ; the ends are rounded and sometimes pointed, especially in those cases where division has taken place. Not

unfrequently there are found forms of very peculiar shape. Some are bent and often club-shaped, and present other irregularities in thickness. That these forms are only modifications of the same organism is distinctly proved by the fact that they are found along with the ordinary forms in one and the same colony when the organism is submitted to plate-cultivation. These forms do not appear to be due to involution, as they occur in fresh cultivations and stain well. Neither were spores observed in these nor in any of the other forms. A very large number of microscopic preparations were made from different cultivations of this organism in order to confirm these observations. (See Plate 20, fig. 2, No. 2*b*.)

In drop-cultivations the bacillus is seen to be non-motile.

Appearance in Cultivations.

Gelatine.—After four days (August 6—10, 1886) the needle-track below presents a slight saw-like growth, whilst on the surface there is a small leaf-like expansion extending on either side of the needle-streak. (See Plate 20, No. 2*a*.) This expansion is of a distinct lemon-yellow colour, with a smooth shining surface. The growth does not extend much on keeping the cultivation longer, and no liquefaction of the gelatine takes place.

Agar-agar.—Forms a moist shining surface-expansion of sulphur-yellow colour, and with a lobular edge. The growth, even in old cultivations, was not found to extend over the whole surface.

Broth.—After nine days (August 7—16, 1886) the liquid is clear, free from pellicle, and has a very slight yellowish deposit at the bottom.

Appearance on plate-cultivation.—The colonies are visible to the naked eye as small white discs after four days, which, on keeping longer, become of a strong yellow colour.

Under a low power ($\times 100$) the colonies are seen to be highly granular, more or less regularly circular in shape, and with an almost smooth edge. (See Plate 20, No. 2*c*.)

4. *BACILLUS PLICATUS.*

Occurrence.—This was found forming a white irregular protuberance on the surface of a gelatine dish which had been exposed to the air in one of the wards of the Brompton Hospital for Consumption.

Microscopic appearance.—Under a high power this is seen to be a very minute bacillus, about $1\frac{1}{2}$ times as long as broad. Usually several bacilli are adherent together, thus forming short threads (see Plate 18, fig. 7, No. 7*b*), the length of which varies from 1.7μ to 5μ .

Seen in drop-cultivations, it was found to be very motile. No spore formation was observed.

Appearance in Cultivations.

Gelatine.—The growth to which this organism gives rise in gelatine is exceedingly characteristic. On the surface there appears along the needle-streak a much crumpled and folded greyish expansion, the peripheral corrugation of which causes the surface to become abundantly pitted and excavated. The growth in the needle-track *below* is much less vigorous than *on* the surface, although in course of time it becomes developed to a considerable extent and has a beaded appearance.

It causes no liquefaction of the gelatine, even in old cultivations. (See Plate 18, No. 7a.)

Agar-agar.—The appearance is very similar to that of the cultivation in gelatine; the surface is, however, of a somewhat more moist texture, and the edge extends in thin fern-shaped expansions over the surface of the agar-agar.

Broth.—After nine days (August 7—16, 1886) the liquid is very slightly turbid, has a dirty-white deposit, and there is a small amount of flocculent matter on the surface; and, adhering to the sides of the tube, this develops later on into a tough irregular pellicle.

Appearance on plate-cultivation.—After four days the colonies appear to the naked eye as small white discs, the larger ones, which have reached the surface, exhibiting an indentation in the centre. As growth proceeds, the centre of the colony remains depressed, whilst the circumference becomes irregularly folded and raised, so that the colony is only attached to the surface of the gelatine by a comparatively narrow pellicle. The substance of the colony is very tough in character, so that the whole growth can be easily removed in its entirety by means of a needle.

Under a low power ($\times 100$) the small colonies have a rough irregular edge varying in shape and degree of roundness. The larger colonies are dark-brown near the edge, but of a lighter shade near the centre; they are very irregular in shape; the contents are finely granular. The different stages of development are exhibited in Plate 18, No. 7a.

5. BACILLUS CHLORINUS.

Occurrence.—This was found as a yellow slowly-liquefying expansion on the surface of a gelatine dish exposed to the air on the spire of Norwich Cathedral. We have found it on numerous occasions to be very prevalent in air.

Microscopic appearance.—Under a high power this is seen to be a very short bacillus, varying from 0.5μ to 1.5μ in length and about half as broad as long; the extremities are rounded. It occurs singly and in short chains. (See Plate 17, fig. 7, No. 7b.) In drop-cultivations only vibratory motion was observed.

Appearance in Cultivations.

Gelatine.—After four days (August 6—10, 1886) the needle-track below exhibits only a very faint growth, whilst on the surface there is a liquefied depression with a lemon-yellow deposit. Liquefaction proceeds slowly, the track of the needle below the surface remaining very faint.

Agar-agar.—Produces a strong, almost uniform, shining surface-growth of a greenish-yellow colour.

Broth.—After nine days (August 7—16, 1886) the liquid exhibits a fine turbidity : there is no pellicle on the surface, but a dirty-yellow deposit on the bottom.

Appearance on plate-cultivation.—On the third day (October 29—November 1, 1886) the colonies appear as greenish shining expansions, rapidly extending on the surface, but remaining small in the depth of the gelatine.

Under a low power ($\times 100$) the larger surface-colonies exhibit very fine granulation, with a thin smooth edge. The smaller colonies have also a smooth sharp edge, with a cloudy interior. (See Plate 17, No. 7a.)

6. *BACILLUS POLYMORPHUS.*

Occurrence.—This was obtained as a small colourless pin-head with radiated rim on the surface of a gelatine dish which had been exposed to the air on the roof of the Science Schools, South Kensington Museum.

Microscopic appearance.—This organism exhibits a great variety of forms, even in cultivations only one day old. In the first place there are seen small fat bacilli, almost like micrococci; then there are longer or more oval individuals, frequently occurring in pairs, and also forming strings of irregular thickness. In these strings there is frequently no division visible, and such an irregular band sometimes reaches 17μ in length. The isolated bacilli are 8μ in length and nearly as wide, whilst when united in chains they appear several times this size.

At first sight this variety of form has the appearance of an impure cultivation. We have, however, found the same variety in examining the contents of single colonies from plate-cultivations of this organism (see Plate 17, fig. 6, Nos. 6b, 6c), and there can, therefore, be no doubt that all these forms belong to one and the same organism. No. 6b was taken from a colony obtained on plate-cultivation, and No. 6c from an agar-tube cultivation. Viewed in drop-cultivations, they appear almost like micrococci, singly and in chains of varying length. Vibratory motion only was observable.

Appearance in Cultivations.

Gelatine.—Slowly forms a surface-growth which is characterised by the regularity of its shape and the minutely serrated nature of its contour. The surface of the growth is smooth and white, but in old cultivations the centre becomes tilted slightly

yellow. (See Plate 17, No. 6*c*.) The needle-track below the surface exhibits a fine saw-like growth, which is more considerable than that of many of the organisms described above.

Agar-agar.—The growth again exhibits a highly serrated edge, but the rate of extension over the surface is more rapid than in the case of the gelatine.

Broth.—After nine days (August 7—16, 1886) the liquid is turbid above, but clear below, and is clothed with a thin cloudy-white pellicle on the surface. There is also a white deposit at the bottom.

Appearance on plate-cultivation.—To the naked eye the colonies are circular and bluish-white, with a small yellow spot in the centre. On the surface of the gelatine they form distinct pin-heads. Under a low power ($\times 100$) the larger surface-colonies are seen to be circular, with an irregular corrugated edge, enclosing coarse granular matter. The central portion is cloudy and surrounded by a distinct ring. (See Plate 17, No. 6*d*.)

The smaller colonies in the depth of the gelatine are very irregular in shape and resemble the corolla of a flower. The contents of the colony is more finely granular than those of the larger surface-colonies, the centre being also clouded. (See Plate 17, No. 6*e*.)

As in other cases, the cultivations were made by inoculating from both kinds of colonies, and the identity of the two proved.

7. BACILLUS PROFUSUS.

Occurrence.—This was found in the air collected on the roof of the Science Schools, South Kensington Museum, producing a beautiful iridescent growth on the surface of gelatine.

Microscopic appearance.—Under a high power it is seen to be a short fat bacillus with rounded extremities. The length reaches about 1.7μ and the width about $.5\mu$. As seen in Plate 18, fig. 2, No. 2*a*, the dimensions of the bacilli are very variable even in one and the same colony (the drawing was made from a preparation taken from a colony). These larger forms are comparatively rare; their length is more than 1.7μ , the width even sometimes reaching that figure.

Viewed in drop-cultivations, they were found to exhibit vibratory motion only, and were seen isolated as well as hanging together in short chains of two, three, and four.

Appearance in Cultivations.

Gelatine.—There is but little growth in the path of the needle *below*, but *on* the surface it frequently extends in a very thin layer which has a beautiful opalescent appearance when viewed by transmitted light.

Agar-agar.—On this medium it forms a much thicker growth, giving rise to a smooth, whitish, lobular expansion, the thinner foliated margin of which exhibits beautiful iridescence by transmitted light.

Broth.—After seven days (August 17—24, 1886) the liquid is clear, excepting the surface, on which there is some thin, granular, floating matter, and at the bottom there is a small amount of whitish deposit.

Appearance on plate-cultivation.—The surface-colonies are seen with the naked eye to form an opalescent expansion of increasing size, with a very irregular contour. (See Plate 18, No. 2*d*.) In the depth of the gelatine, on the other hand, the colonies appear as grey dots. Under a low power ($\times 100$) the surface-colonies exhibit a dense centre, surrounded by a very thin and granular expansion having a highly irregular contour. (See Plate 18, No. 2*b*.) The drawing represents a colony in which this surface excrescence is commencing. No. 2*c* represents a colony in the depth of the gelatine. Viewed against the light, these surface-colonies are of a beautiful azure-blue colour.

8. BACILLUS PESTIFER.

Occurrence.—This was found forming a small white expansion on the surface of a gelatine-dish which had been exposed to the air in a garden near Hughenden, Bucks.

Microscopic appearance.—Under a high power this is seen to be a large thick bacillus about 3.4μ in length and from $.8\mu$ to 1.7μ in thickness; the length is difficult to determine, owing to the formation of threads, which are frequently of great length, extending far beyond the field of the microscope, and giving rise to winding vermiform figures. (See Plate 19, fig. 7, No. 7*b*.)

Viewed in drop-cultivations, the bacilli are seen to exist singly, in pairs, threes and fours, &c., up to exceedingly long threads. Their movement is slow and undulating, the single bacilli exhibiting most motility. It also forms non-motile tangled masses, but in no case was spore-formation observed. In Plate 19, No. 7*c*, which is drawn from a drop-cultivation, the arrangement of the bacilli in smaller groups is shown. Although we have examined a very large number of preparations of this organism, both in young and old cultivations, in gelatine, agar-agar, and broth, we have never observed any spore-formation.

Appearance in Cultivations.

Gelatine.—On the surface it produces an almost colourless feathery expansion, which causes slow liquefaction of the gelatine.

Agar-agar.—Commences by forming a grey-white smooth surface-growth, which rapidly extends over the agar; the surface-growth sometimes becomes very much wrinkled, like that of the *Bacillus subtilis* (see below), but it has a more moist and shining appearance than the latter, and is of a grey, transparent, almost colourless hue. The wrinkles are very highly convoluted and twisted. (See Plate 19, No. 7*a*.)

Broth.—After four days (August 31—September 4, 1886) the liquid is slightly

turbid, free from pellicle, and has a small quantity of white deposit at the bottom. Even after thirteen days there is only a thin film on the surface, which falls to the bottom on shaking, and there is very little deposit.

Appearance on plate-cultivation.—After two days the colonies appear to the naked eye only as white specks, but seen with a low power ($\times 100$) those on the surface exhibit a very irregular contour, consisting of branchings into the surrounding gelatine of threads; the interior of the colony has the appearance of being composed of threads closely packed together; as they develop further, the centre becomes very dark and cloudy, but the edge remains very light, and thus much resembles a crystal branching out in feathers into the surrounding gelatine; after five days the feathery contours can be seen with an ordinary magnifying glass. In the depth the colonies appear compact and almost circular. (See Plate 19, No. 7d.)

In all cultivations this organism gives rise to a most disagreeable odour, somewhat resembling that of putrid blood.

9. *BACILLUS LÆVIS*.

Occurrence.—This was found forming a yellowish-white liquefying growth on the surface of a gelatine-dish which had been exposed to the air in one of the wards of the Brompton Hospital for Consumption.

Microscopic appearance.—Under a high power this is seen to be a bacillus the average length of which is 1.7 to 2.5μ , and it is about 5 times as long as broad; the ends are distinctly rounded. It occurs singly, often in pairs, and occasionally in threads. It gives rise to spores which are nearly as long as the bacillus itself, but more oval in shape, and which exhibit the characteristic highly refractive appearance of spores in general. All the well-known forms of *Bacillus subtilis* were observed, including the thickened form, only on a much smaller scale, and the threads being considerably shorter.

In preparations made from the surface of agar-agar cultivations frequently nothing but spores were visible. Whilst the bacillus is readily stained with any of the ordinary aniline colours (gentian-violet, &c.), the spores prove refractory as usual.

In Plate 19, fig. 6, No. 6c, the preparation was made from a gelatine-cultivation of ten days' age. In No. 6b the appearance is shown when a preparation is made from a colony after three days' growth. Tube-cultivations started from such colonies yielded in course of time all the various forms represented in No. 6c.

In drop-cultivations the bacilli are seen to be exceedingly motile, occurring singly, in pairs, and occasionally in threads; subsequently stationary masses of bacilli make their appearance, and shining spores are visible.

Appearance in Cultivations.

Gelatine.—After four days liquefaction has commenced at the top of the needle-track, forming a round depression, the bottom of which is filled with a white cloudy liquid. After nine days the liquefaction has extended across the whole tube to a depth of half-an-inch. The liquid is turbid and has a tough, greyish, wrinkled pellicle upon its surface and a flocculent deposit at the bottom. The lower part of the needle-track does not exhibit much alteration even at this stage. Ultimately the whole contents of the tube becomes liquid. (See Plate 19, No. 6a.)

Agar-agar.—The growth is but little characteristic. It forms a moist, shining, greyish-white surface expansion, which rapidly extends over the whole agar-agar.

Broth.—After nine days (August 7—16, 1886) the liquid is turbid near the surface and clear below; there is a dirty-white flocculent deposit at the bottom, and a thin granular pellicle on the surface. Subsequently the liquid becomes clear, the pellicle remaining on the surface.

Appearance on plate-cultivation.—In three days the colonies are visible to the naked eye as small white dots, the surface-colonies exhibiting a slight flocculence, which indicates the commencement of liquefaction; as the colonies increase in size, liquefaction of the gelatine slowly proceeds.

Under a low power ($\times 100$) the colonies in the depth of the gelatine are seen to have a smooth edge, which is irregular in shape and encloses granular contents.

The surface-colonies exhibit a very fine thin film of irregular shape, extending from a small centre, indicating the spot where the colony first reached the surface and began to liquefy. (See Plate 19, No. 6d.)

The characteristic differences between this organism and *Bacillus subtilis* will be pointed out after the latter has been fully described.

10. *BACILLUS CEREUS*.

Occurrence.—This was found producing a large liquefying colony on a gelatine-dish which had been exposed to the air in a cow-shed.

Microscopic appearance.—The bacilli are from 3.4 to 12μ in length. There are also seen thickened forms about 3.4μ long and 1.7μ wide. The ends of the bacilli are generally slightly rounded, whilst some are almost quite square. The bacilli form threads which are very variable in length, some being composed of ten segments or more.

Spore-formation was also observed as seen in the Plate. (See Plate 20, fig. 3, No. 3a.)

In a drop-cultivation the following changes were observed to take place:—

When examined directly after inoculation from an agar-agar cultivation, there were visible isolated bacilli, many of which contained a single spore, and free spores were also present; there was practically no movement taking place. Within 12 hours

there were numerous very motile bacilli, generally isolated, but occasionally forming longer threads.

After 24 hours the bacilli were perfectly motionless, generally in pairs or in threads of three and four. After 48 hours the bacilli were still stationary, and there was abundant spore-formation, each segment exhibiting a shining oval spore in its interior. As the cultivation increased in age the threads were gradually broken up and the spores liberated. The free spores exhibit vibratory movement.

Appearance in Cultivations.

Gelatine.—The mode of growth essentially resembles that of the *Bacillus subtilis* in this medium, the only difference being that it causes more rapid liquefaction of the gelatine than the latter.

Broth.—Growth practically identical with that of *Bacillus subtilis* in this medium.

Agar-agar.—The growth in this medium presents a marked difference to that of *Bacillus subtilis*. It forms a moist, grey-white, smooth, wax-like expansion, which rapidly extends over the surface of the agar-agar. Even in very old cultivations no wrinkling, but only slight granulation of the surface, takes place.

Appearance on plate-cultivation.—Owing to the exceedingly rapid liquefaction of the gelatine which this organism causes, it is necessary to examine the plates within 24 hours of their being poured, in order to observe the first appearances presented by the colonies.

We have examined a number of plate-cultivations of this organism, but the following description will serve to illustrate the progressive development of the colonies.

After keeping the plate at 18–20° C. for 18 hours, the colonies were just visible to the naked eye as small white dots, no apparent liquefaction having yet set in. Under a low power ($\times 100$) the colonies appear as round or oval woolly masses having a finely spinose edge, from which, in many cases, long whip-like and spirally-coiled threads extended into the surrounding gelatine. Some of the colonies, on reaching the surface, gave rise to highly-irregular filamentous growths consisting of bands of fine threads, as subsequently described and drawn in the case of the colonies of *Bacillus subtilis*. (See Plate 20, fig. 5, Nos. 5e, 5f.) These filamentous surface-growths sometimes appear as though they were not derived from any colony of the usual kind, but had arisen quite independently; this appears to be due to the colony from which they proceed having been situated very near the surface, and having only attained very insignificant dimensions before reaching it; and, having once arrived there, the growth on the surface is enormously more rapid than in the depth, and soon produces liquefaction. Other colonies, again, situated in the depth of the gelatine, exhibit a more uniformly spinose contour, as seen in Plate 20, fig. 3, No. 3b.

After 24 hours the colonies had considerably increased in size, being very apparent to the naked eye, although active liquefaction had not yet set in. Under a low power

($\times 100$) the whip-like extensions noticed above had enormously increased, the greater number of the colonies having the appearance presented in Plate 20, fig. 3, No. 3c; others, again, like that shown in Plate 20, fig. 3, No. 3b; and others partaking of the character of both these, as shown in Plate 20, fig. 5, No. 5f. After 36 hours the colonies had further increased in size, and in many cases the whip-like extensions had become much thickened; in some colonies these gave rise to a star-fish appearance, which is easily visible to the naked eye.

We have established beyond doubt that all the above forms of colony are derived from one and the same organism, inasmuch as we have repeatedly prepared plates by inoculation from single colonies and again obtained colonies of the same diversity in appearance.

11. BACILLUS SUBTILIS. (Hay Bacillus.)

Although this micro-organism has become classical through the great care with which it has been described by numerous authorities, including COHN, KOCH, KLEIN, FLÜGGE, and many others, it is only recently that the appearances to which it gives rise on plate-cultivation have been recorded (EISENBERG, *loc. cit.*; FLÜGGE, *loc. cit.*). We have had occasion to carefully examine the appearances produced by this organism in order to compare them with those resulting from some of the organisms described above. For the purposes of this comparison, we have employed a cultivation which was obtained by one of us from Dr. KOCH's laboratory in Berlin.

Microscopic Appearance.

The single bacilli vary in length from 1.7μ to 6.8μ , and are about 1.7μ in width; the ends are slightly rounded, but sometimes nearly rectangular. Prior to spore-formation the bacilli become thicker and more square (see Plate 20, fig. 5, No. 5c) and, as described in the case of *B. cereus*, these thicker forms present a very different appearance to the ordinary bacilli. The bacilli also grow into threads, which are frequently of great length. The spores, which are to be seen in all but the newest cultivations, have a length of about 2.5μ , and are about 1μ in width; they are oval, and present, as usual, a bright and shining appearance, which, together with their property of not staining with aniline colours, renders them easily distinguishable from the bacilli. In Plate 20, fig. 5, Nos. 5c and 5g, these various forms are represented; thus in No. 5g are the ordinary bacilli, also the thickened bacilli, also bacilli containing spores; whilst in No. 5c a thread is shown composed of numerous segments, also a similar thread which has become thickened and exhibits a spore in each segment.

Viewed in drop-cultivations, the isolated bacilli are seen to be very motile, but there are also stationary masses of bacilli which are non-motile. Subsequently threads and spore-formation are observable, as previously described in the case of *B. cereus*.

Appearance in Cultivations.

Gelatine.—The growth gives rise in the course of a few days to liquefaction of the gelatine in the form of a long funnel, the lower part of which throws out feathery lateral extensions into the adjacent gelatine. (See Plate 20, fig. 5, No. 5a.) Soon the liquefaction extends across the tube at the surface, and ultimately involves its whole contents, a tough white pellicle forming on the surface, the liquid below becoming clear, and a large quantity of flocculent matter becoming deposited at the bottom.

Agar-agar.—The growth rapidly extends over the surface as a white opaque expansion, which soon assumes a dry appearance and becomes copiously wrinkled and puckered. (See Plate 20, fig. 5, No. 5b.)

Broth.—Grows rapidly, rendering the liquid turbid and giving rise to a white deposit at the bottom, and forming a pellicle on the surface which gradually increases in thickness and tenacity.

Appearance on plate-cultivation.—The colonies become visible to the naked eye in about two days' time as small white dots when beneath, whilst on the surface they exhibit a very small liquefied circle of a greyish hue.

Under a low power ($\times 100$) the colonies in the depth of the gelatine are seen to have an irregular contour, with short spinose extensions in parts of the circumference, and the interior of each colony has a wavy structure, as if composed of coiled threads. (See Plate 20, fig. 5, No. 5d.) As the colonies increase in size the internal structure becomes less defined, whilst the circumference becomes uniformly spinose (See Plate 20, fig. 3, No. 3b.)

In the early stages (about after two days' growth) the surface of the gelatine presents in places small cloudy expansions, which, when viewed under a low power ($\times 106$) exhibit a most characteristic appearance, which seems to have hitherto escaped observation, consisting of a highly-irregular figure (see Plate 20, fig. 5, No. 5e), composed of parallel bands of fine threads arranged in a much-contorted pattern. This appears to be the form assumed by the colonies on first reaching the surface of the gelatine, for, on further preserving a plate exhibiting a number of such "thread" colonies, in the course of a day or two their appearance will be found to have entirely changed, their place being taken by a liquefied surface, the margin of which exhibits the usual spinose character first described. In Plate 20, fig. 5, No. 5f, an ordinary spinose colony in the depth is seen to be breaking out into a thread-expansion where it has reached the surface.

Tubes both of gelatine and agar-agar were inoculated from both varieties of colony, each giving rise to the same characteristic appearances already described. Plates were again prepared from these separate cultivations, and both varieties of colony obtained from each cultivation.

It thus appears that *Bacillus subtilis* as well as *B. cereus*, described above, both form colonies of several different types, the form of which depends upon the position in the gelatine-film of the bacilli from which they are derived.

In the first place there are compact colonies in the depth of the gelatine, which soon show small spinose or hair-like extensions from the periphery. These extensions increase in thickness, but remain fairly uniform in length, and ultimately the colony produces a liquid circle in the gelatine, and the periphery of this circle has also a finely spinose appearance. This appears to be the only form of colony which has been described by other observers. The appearance of these colonies may be compared to that of a "*crown of thorns*."

In a modification of the first class of colony, which appears to arise when the plates are incubated at a somewhat higher temperature, the hair-like extensions from the compact colony in the depth are much longer and irregular, often spiral and twisted, or resembling the lash of a whip. The formation of these longer extensions is probably accounted for by the smaller resistance offered by the gelatine at the higher temperature. These likewise ultimately produce liquefied colonies which do not differ in appearance from the liquefied colonies of the first class. This second class may be designated *whip-colonies*. These whip-like colonies appear to be especially characteristic of *Bacillus cereus*. (See Plate 20, fig. 3, No. 3c.)

Thirdly, there are found surface-growths of very remarkable appearance, and consisting of parallel bands of threads meandering over the surface of the gelatine in the most capricious manner, and frequently expanding into coils. These surface-growths are often quite independent of any compact colony, their production being, as far as we can see, due to the original individual organism or group of organisms from which they have sprung being situated so near the surface of the gelatine that the first out-growth, having promptly reached the surface, has there grown out in free contact with the air with enormous rapidity. Thus in very young plates these surface-growths are already found of enormous dimensions compared with the compact colonies in the lower strata of the gelatine. This third class may be fitly named *meander-colonies*. They also give rise to liquefaction, the edge of the liquefied portion being similar to that of the liquefied colonies of the other two classes. We have found these meander-colonies in the case of the *Bacillus cereus* as well as in that of *Bacillus subtilis*.

In Plate 20, fig. 4, we have represented a colony of the *Bacillus anthracis* which presents many points of resemblance to those of the *Bacillus subtilis*, more especially to the "whip" and "meander" modifications.

Points of Distinction between B. lævis, B. cereus, and B. subtilis.

From the descriptions which we have given above it will be seen that these three micro-organisms resemble each other very closely in many points; they can, however, be sharply distinguished on the following grounds:—

Liquefaction of gelatine.—*B. cereus* causes by far the most rapid liquefaction of the gelatine; *B. subtilis* stands second in this respect, the liquefaction being very

decidedly less rapid; whilst *B. lævis* possesses the power of liquefying gelatine to a much less extent than either of the other two.

Agar-agar cultivations.—*B. subtilis* is very sharply distinguishable from the other two by the property which it possesses of imparting a characteristic wrinkled appearance to the surface of the agar-agar.

Colonies on gelatine plates.—The colonies produced by *B. lævis* are, as has been shown above, very different from those of the other two, whilst we have not been able to establish any substantial difference between the colonies of *subtilis* and *cereus* beyond the difference in the rate of liquefaction, already referred to, and the longer and more spiral form of the whip-like extensions which we have constantly observed to be characteristic of *B. cereus*.

Microscopic appearance.—In this respect, again, *B. lævis* exhibits a marked difference from the other two, its dimensions being very decidedly smaller.

12. BACILLUS (MICROCOCOCCUS) PRODIGIOSUS.

This organism was first described by COHEN as a micrococcus, but is now generally regarded as a bacillus.

We have met with this organism both in air and water, but we have especially studied it from a cultivation obtained by one of us from Dr. KOCH's laboratory in Berlin.

Microscopic appearance.—The cells are rather longer than broad, the largest forms being about 1.7μ in length and about 1μ in width; they are frequently found hanging together in pairs. More distinctly bacillar forms have been described by CORNIL and BABÈS ('Les Bactéries,' 1886, p. 141) as occurring in broth-cultivations. We can fully confirm these observations, having ourselves seen them in drop-cultivations, and, what is more convincing, in single colonies. (See Plate 20, fig. 1, No. 1c.)

Appearance in Cultivations.

Gelatine.—Grows very rapidly, liquefying the gelatine in the form of a conical sack (see Plate 20, No. 1b) which soon extends across the tube at the top and, gradually passing downwards, involves the whole tube. The liquid formed is very turbid, with an abundant flocculent deposit of an intensely crimson colour. Near the surface there is generally seen adhering to the glass a thin layer of still darker red colouring matter which has the peculiar fluorescence of an aniline colour when in a concentrated state.

Agar-agar.—Grows very rapidly over the surface, producing a deep, blood-red, smooth and shining expansion, the colour being only developed on the surface. (See Plate 20, No. 1a.)

Broth.—Grows rapidly; rendering the broth turbid, and producing in the first

instance a white deposit, which later on becomes of a pinkish colour, whilst at the surface the colour is visible much sooner.

Appearance on plate-cultivation.—After two days the colonies are seen to the naked eye as circular depressions, each having a red centre. Under a low power ($\times 100$) the less-developed colonies in the depth of the gelatine are devoid of red colour; they are finely granular, with a very irregular contour. (See Plate 20, No. 1*d*.) The surface-colonies, on the other hand, have a distinctly red nucleus surrounded by a very thin and finely granular brownish growth having a very irregular contour. (See Plate 20, No. 1*d*.) No. 1*e* represents the appearance of the colonies to the naked eye.

III.—SACCHAROMYCES.

We have found two varieties of *Saccharomyces* in the air, the one colourless, and the other producing a red pigment. They are both of very frequent occurrence.

1. *SACCHAROMYCES LIQUEFACIENS*.

This organism produces very characteristic star-shaped liquefying colonies on gelatine-plates which have been exposed to the air.

Microscopic appearance.—The individual cells are oval and 7 and even 9μ in length, and from 3 to 5μ in width. They appear only to bud from the apex, and frequently there are twin buds from the same apex of the parent cell; occasionally they are seen hanging together in long strings. (See Plate 19, fig. 1, No. 1*d*.)

Appearance in Cultivations.

Gelatine.—After a few days the needle-track below the surface exhibits small feathery centres in its lower portion, whilst in its upper portion the track is continuous and throws out hair-like lateral extensions of increasing length into the adjacent gelatine. On the surface there is a depression filled with white, cloudy, liquefied matter. Later on, the liquefaction extends across the tube, and ultimately involves the whole of the gelatine. (See Plate 19, No. 1*a*.)

Agar-agar.—Forms a shining surface-growth exhibiting radial marking from the central puncture. The surface is *very* faintly pink in colour. The track of the needle beneath the surface exhibits beautiful feathery lateral extensions. (See Plate 19, No. 1*b*.)

The growth both in gelatine and agar-agar is exceedingly characteristic.

Broth.—After nine days (August 7—16, 1886) the liquid is clear, with a dirty-white deposit at the bottom.

Appearance on plate-cultivation.—The colonies when young appear as small cloudy centres to the naked eye. As they increase in size they assume a star-shaped appearance, the rays of which gradually extend and liquefy the gelatine in a highly characteristic manner.

Under a low power ($\times 100$) the branches of the star are seen to be composed of the individual cells closely compressed together in packets. The appearance is shown in Plate 19, 1c. No 1e represents the naked-eye appearance of a colony.

In its microscopic appearance this saccharomyces appears to correspond with the description of *S. apiculatus*, but the behaviour of the latter in cultivations has not been described.

2. SACCHAROMYCES ROSACEUS.

We have found this on gelatine-plates which have been exposed to the air. It forms small pink pin-head colonies on the surface of the gelatine.

Microscopic appearance.—The cells are long, oval, about 8.5μ long and 3.5μ in width. They appear to bud from the extremities only, and there are frequently two twin buds adhering to the same extremity of the parent cell. They are sometimes seen hanging together in chains of four. (See Plate 19, 2b.)

Appearance in Cultivations.

Gelatine.—It forms a shining pink expansion on the surface, there being but little growth in the track of the needle below.

Agar-agar.—On the surface it forms a smooth shining growth of a beautiful rose colour. The growth exhibits lines of darker tint radiating from a lighter centre (see Plate 19, 2a). There is no colour and but little growth in the needle-track below the surface.

Broth.—After six days the liquid is clear, with a dirty-pink deposit at the bottom, which later on becomes of a bright-pink colour, the liquid remaining clear.

Appearance on plate-cultivation.—The colonies are seen to the naked eye as shining pink pin-heads, which do not cause liquefaction of the gelatine. Under a lower power ($\times 100$) the individual cells composing the closely packed masses of which the colonies consist can be distinctly seen, especially in the case of the surface colonies. (See Plate 19, 2c.)

IV.—MOULD PRODUCING A BROWN PIGMENT.

This was found by us producing a dark-brown pigment on a gelatine-plate which had been exposed to the air on Norwich Cathedral.

Microscopic appearance.—It forms a tangled network of branching filaments without visible division; in thickness it goes up to $.8\mu$. In Plate 18, fig. 1, No. 1d, the filaments are shown magnified about 600 times. No spore-formation has been observed.

Appearance in Cultivations.

Gelatine.—After a few days the needle-track below the surface exhibits small, isolated, flocculent centres. The surface is depressed and exhibits greyish flocculent

plaques fringed with brown, which shades off into the surrounding gelatine.* As the age of the cultivation increases, the brown colour becomes more intense and gradually extends throughout the tube. The gelatine becomes slowly liquefied, the fluid being of the same dark-brown colour. (See Plate 18, No. 1*b*.)

Agar-agar.—The surface of the agar becomes covered with flocculent greyish plaques around which the brown colour is formed, the latter extending gradually into the depth of the tube. (See Plate 18, No. 1*a*.)

Broth.—The appearance is very characteristic. Numerous small, isolated, spherical tufts make their appearance throughout the liquid, adhering to the sides and bottom of the tube. (See Plate 18, No. 1*c*.) The liquid, which is quite clear, becomes of a deep sherry colour.

Appearance on plate-cultivation.—The colonies appear as cream-white disks, each of which is surrounded by a cloud of brown colouring matter extending over a distance many times the diameter of the colony.

Under a low power ($\times 100$) the colony is seen to consist of fine branching threads radiating from a central tangled mass.

* This surface-growth is of such a tough nature that it is with some difficulty that a portion can be removed with a platinum-needle.

XII. *On the Organisation of the Fossil Plants of the Coal-Measures.*—PART XIII.
Heterangium Tiliæoides (WILLIAMSON) and *Kaloxylon Hookeri*.

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[PLATES 21—24.]

IN 1872 I described ('Phil. Trans.,' 1873, "On the Organisation of the Fossil Plants of the Coal-Measures—Part IV."), under the name of *Heterangium Grievii*, one of the most interesting of the various plants which I discovered in the Burntisland deposit of Carboniferous limestone at Kinghorn. More recently we have obtained, from the Halifax beds, a very distinct plant, which, though differing in many important features from *H. Grievii*, resembles it so closely in others that I propose to include it in the same genus, under the name of *Heterangium Tiliæoides*. Its central medullary axis, A, differs in no conspicuous manner from that of *H. Grievii* when transverse sections of the two are compared.

Fig. 1 represents such a section enlarged 15 diameters, the medullary portion being seen at A, and a similar section is shown at fig. 2, A; whilst fig. 3 represents a small portion of a section enlarged 103 diameters. From the latter of these sections it will be seen that this medullary axis consists of irregular clusters of vessels or tracheids, *b*, the intervals between which are filled with ordinary parenchyma, *a*. The vessels near the periphery of this axis are smaller than those constituting the more central bundles—a feature best shown in fig. 2, *b'*. The character of these vessels will be referred to immediately.

Closely surrounding the medullary axis is a well-defined exogenous zone, B. This consists of a circle of vascular wedges, *c*; each wedge being composed of a number of laminæ which spring from a cluster of the smaller vessels, *U'*, which form the periphery of the medullary axis. At their medullary extremities the more external of the laminæ, fig. 4, *d*, composing each wedge, bend inwards towards its more central ones, so that each bundle represents an obtuse wedge-shaped group of such laminæ separated from a similar group on either side of it by a primary medullary ray (figs. 1, 2, and 4, *g*). The vessels of each lamina increase in size from within outwards, and between each one or two laminæ (fig. 4, *d*) we find secondary medullary

rays (fig. 4, *h*). A cambium layer obviously existed along the lines *i, i*, of figs. 1, 2, and 4.

Externally to the xylem zone, B, we have what constitutes the most characteristic feature of this plant, viz., a true phloem zone, C. Each of the phloem-masses, *k*, corresponds in breadth to that of the xylem wedge to which it belongs. In many of these phloem-masses their component tissues are preserved only in a compressed or disturbed condition; but in the figure, Plate 21, fig. 4, *k*, we discover transverse sections of cells and tubular structures of various sizes, which exhibit a strong tendency to arrange themselves, especially at the inner portion of the phloem, in radial lines. In this example the xylem portion of the bundle extends laterally from *g* to *g*. The phloem, *k*, extends upwards and outwards in this figure until its peripheral margin comes in contact with the inner one, *p*, of the true cortex. This illustration is enlarged 48 diameters.

The large primary medullary rays which separate the xylem portions of contiguous fibre-vascular wedges or bundles now expand, as they proceed outwards, into magnificent primary phloem rays (Plate 21, fig. 1; Plate 22, fig. 2; and Plate 21, fig. 4; *n, n*). The Botanist will at once recognise the remarkable resemblance of these conspicuous phloem rays to those occupying a corresponding position in the phloem of the common Lime, which resemblance has led me to adopt the specific name of *Tiliacoides* for this species of *Heterangium*. Each of these rays, as seen in transverse sections of a stem or branch, fig. 4, *n*, becomes broadly trumpet-shaped as it proceeds outwards from the extremity of the medullary ray, *g*, of which it is an extension. The large, more or less cubical, parenchymatous cells composing it are arranged in irregularly curved lines, the concavities of which are directed towards the medullary axis. Externally these cells merge with those of the inner cortex, *p*. Secondary and smaller phloem rays, extensions of the secondary medullary rays, *h*, can also be traced in all the transverse sections. They are sufficiently distinct in the phloem, *k*, of the isolated wedge, *c*, of fig. 5.

The cortex, D, is separable into two layers, an inner one, *p*, and an outer one, *r*. The inner one (figs. 1, 2, and 4, *p, p*) consists of a comparatively uniform parenchyma which presents no special features of interest. The outer cortex, *r, r*, transverse sections of which are seen in figs. 1, 2, and 6, is somewhat more complicated. It consists of parenchymatous cells of variable forms and sizes. As seen in figs. 2 and 6, the inner cells, *r*, are much larger than the outer ones, *r', r'*. The orifices, *w, w*, of fig. 6 are merely worm-borings and not normal cavities. Two important additional structures exist in transverse sections of the outer cortex. One of these consists of transverse sections of ascending vascular bundles grouped in pairs. In Plate 21, fig. 1, two imperfect examples of these twin arrangements are seen at *u* and *u'*; two such bundles are enlarged $51\frac{1}{2}$ times in Plate 22, fig. 7, *u, u*, and a similar pair of still larger ones is enlarged 20 diameters at *u, u*, of fig. 8.

The second structure seen in transverse sections of the outer bark consists of

masses, of unequal size and shape, of sclerous cells. Examples of these are seen at *t*, *t*, in Plate 21, fig. 1, Plate 22, fig. 2, Plate 21, fig. 6, and Plate 22, fig. 8. The true relations of these masses of sclerenchyma will be seen on examining the vertical sections of the cortex.

Plate 3, fig. 9, represents a vertical section made radially, and passing through the medullary axis at A, the exogenous xylem zone at B, the phloem zone at C, and the innermost border of the inner cortex at D. This section has passed from above downwards in a slightly oblique manner, so as successively to intersect five of the vascular laminæ, *d*, and the intervening secondary medullary rays, *h*, of the fibro-vascular bundle through which the section passes. Each of these alternating layers comes successively into view as we pass from the upper to the lower margins of the Plate. As is so often the case with the fossil Cryptogams, we find the cubical parenchymatous cells of the medullary axis, Plate 23, fig. 9, *a*, *a*, exhibiting a tendency to arrange themselves in vertical lines and with approximately rectangular horizontal septa. The vessels or tracheids, *b*, of this axis vary much in diameter. As already pointed out, those at its periphery and in contact with the inner surface of the xylem cylinder are very small; but the largest of those seen in the isolated bundles (Plate 21, fig. 3, *b*) have a mean diameter of at least $\frac{1}{100} = \cdot 001$ of an inch. More externally we have the vessels of the exogenously developed xylem, the laminæ of which are arranged in parallel radiating series. Their innermost vessels (Plate 23, fig. 9, *e*) are much smaller than the more external ones, *e'*, of the same lamina. The latter have a mean diameter of from $\frac{1}{1200} = \cdot 0008$ to $\frac{1}{1600} = \cdot 00062$.

Alternating with these laminæ of vertically disposed vessels, we have, at Plate 23, fig. 9, *h*, *h*, the secondary medullary rays composed, in these radial sections, of horizontal lines of cells arranged in a mural manner. In tangential sections of this exogenous xylem we find each of these secondary rays composed of one or two vertical series of cells, much compressed laterally. At C we obtain a radial section through the phloem zone of the stem. Here, again, we find numerous long, narrow, vertically arranged tubes (fig. 9, *l*) alternating with murally disposed masses of cells, *n*, constituting the primary or secondary phloem rays. The tubes have a mean diameter $\frac{1}{3100} = \cdot 00031$ of an inch. Of course it is to be presumed that these tubes may be regarded as representatives of the sieve-tubes of the higher Phanerogams, but I can detect in them no traces of transverse septa, or of any structural peculiarities justifying my affirming that they are actually sieve-tubes. It must be remembered that we are equally unable to verify the identity of the similar tubes in the phloem of the *Selaginellæ* with the true sieve-tubes of Ferns and of the Phanerogams.*

Plate 21, fig. 10, represents a tangential section of the phloem of our plant. We here see that the tubes, *l*, just described, display a tendency towards an undulating arrangement, the spaces between the curves of which enclose horizontally disposed lines of cells. I have already called attention to the fact that transverse sections

* See DE BARY, 'Comp. Anatomy of Phanerogams and Ferns' (Engl. Transl.), p. 182.

like Plate 21, fig. 4, C, seem to indicate some disposition on the part of these phloem tubes to arrange themselves in radial lines.

Radial sections of the inner cortex (Plate 23, fig. 9, *p*) exhibit no peculiarities in the form and arrangement of its parenchymatous cells requiring special notice, except one shortly to be referred to. It is otherwise with the outermost cortex *r*, of part of which a longitudinal section, enlarged 31 diameters, is shown in Plate 22, fig. 11. The peripheral layer is wanting in this preparation. It shows, however, at *r*, *r*, the coarse parenchyma seen in the section, Plate 22, fig. 2. It also exhibits three thick bands of sclerenchyma (Plate 22, fig. 11, *t*, *t*, *t*) corresponding to the detached patches of the same tissue seen in figs. 1, 2, and 8. The cells forming these horizontal bands have a mean diameter of from $\frac{1}{1800} = \cdot 00062$ to $\frac{1}{2000} = \cdot 0005$ of an inch. One longitudinal section in my cabinet exhibits one of the twin vascular bundles already described (fig. 8, *u*, *u*) passing upwards and outwards through the cortex.

Plate 23, fig. 12, is a tangential section through the exogenous xylem of a stem or branch which is giving off a true branch, *w*. We have the xylem zone at B, a little of the phloem at C; the inner cortex at *p*, and the outer cortex at *r*, with three of the horizontal masses of sclerenchyma at *t*, *t*, *t*. The section has not been made exactly parallel with the axis of the main stem; hence it has cut obliquely through the considerable branch at *w*. This branch has evidently originated in a tortuous deflection of the xylem vessels, which have only attained to their normal arrangement in regular radiating laminæ in the more external semi-diameter of the branch. The complete development of these radial vascular laminæ would only be attained at a higher point of the branch where it became entirely free from the parent stem. The identical development of a similar branch of *Kaloxylon Hookeri* is shown in Plates 6 and 7, figs. 32, 33, and 34 of my Memoir, Part VII. ('Phil. Trans.,' 1876). The branch, *w*, of fig. 12 is invested by an imperfectly preserved layer, *p'*, of the inner cortical zone. The figure is enlarged 13 diameters.

The resemblance in the origin of the branch just described to that of *Kaloxylon Hookeri* is also further sustained by the specimen represented in Plate 22, fig. 13. In figs. 32 and 33 of the *Kaloxylon* referred to above, the branch is seen to be given off opposite to a large primary medullary ray; the two vascular wedges bounding that ray on its two sides contributing equally their supplies of vessels and cells to the formation of the branch. Fig. 13 exhibits identical conditions. In it we have the large primary medullary and phloem ray, *n*, bounded on its two sides by the lateral vascular laminæ of the two vascular wedges, *c*, *c'*. A mass of tracheids and vessels, *e*, *e'*, is being given off from the peripheral angle of each of these two wedges; these meet beyond the outer boundary of the primary phloem ray, *n*, where they unite to form the rudiments of the branch, *w*. The abundance of short tracheids at this point reminds us of the similar development of these elements where roots or branches are being given off by the stems of living vascular Cryptogams.

There yet remains to be considered the character of the vessels and tracheids which

enter so largely into the composition of this stem. In the twin bundles (Plate 22, figs. 7–8), going off to what, both in this plant and the allied *Heterangium Grievii*, I presume to have been petiolate leaves, the vessels are mainly of the barred or spiral type represented on Plate 22, fig. 14. In the other vascular organs they appear, as represented in fig. 15, to be reticulated; but more careful examinations, aided by higher microscopic powers, show that all these vessels are furnished with the bordered pits seen in Plate 21, fig. 16, the canals of which are all narrow, oblique, and parallel to one another. In the larger proportion of these vessels and tracheids the canals alone remain visible, as in Plate 22, fig. 17; but in fig. 18 we see the transition from the one form to the other in the same tracheid or vessel. At *e* we have in each pit both the central canal and its investing areole; but at *e'* all the areoles have disappeared, I presume during mineralisation, the oblique canals alone remaining; but that each canal was originally surrounded by its own areole is unquestionable.

One more curious feature is presented by this plant. In Plate 22, fig. 5, we have the exogenous xylem zone at B in its normal position relatively to the medullary axis A. But the two fibro-vascular bundles or wedges *c*, *c'*, are being pushed outwards by a mass of parenchyma, *z*, which has developed on the inner side of the two bundles separating them from the medullary axis.

It will further be noticed that each of these bundles carries away with it, on its inner side, a cluster of the medullary vessels *b*, *b*, suggesting the probability that the cellular mass, *z*, has really been an outgrowth from the medullary cells, since it must have originated from the central side of the two clusters of medullary vessels *b*, *b*, which it has been instrumental in pushing outwards. I discover no clue to the destination of these displaced fibro-vascular bundles; but they remind us of somewhat similar conditions seen in the stems of *Lyginodendron Oldhamium*.

Fig. 19 represents a cluster of cortical cells, enlarged 187 diameters. Cells in this condition are extremely abundant in the cortical layers. At the first glance they look like cells with thickened cell-walls, but they are constantly found in close association with cells belonging to the same parts of the plant, the walls of which exhibit no such thickening, hence the effect is possibly due to some condition of mineralisation which has produced deposits of various amounts within the cell-walls.

The next plant requiring further notice is the *Kaloxylon Hookeri*, which I first described in my Memoir, Part VII.* The specimens described and figured on Plates 5, 6, and 7 of that memoir were all obtained from one of the Oldham localities, and, as is not seldom the case with the fossil plants from that quarter, their cellular structures are ill-defined through defective mineralisation. But for several years past we have obtained examples of a *Kaloxylon* from the Halifax beds which are in a very different condition. These specimens long ago showed that the cortical zone possessed peculiarities of structure which the Oldham examples had not revealed to me; but on re-examining those examples, guided by the light thrown upon them by those

* 'Phil. Trans.,' vol. 166, 1876.

from Halifax, I soon found that the cortical tissues of the two forms had been identical.

At the same time, the Halifax specimens had peculiarities of their own which seemed difficult of explanation. The forms most generally met with at Oldham were of the type represented in my earlier memoir by the two figures, Plate 5, figs. 23 and 26, in which six large exogenously developed vascular wedges radiated from what appeared to be a central vascular axis. At that time I was unable to discover any cellular tissue in that axis; I have since obtained three or four examples like those just referred to, from Halifax, one of which is represented in Plate 23, fig. 20, of the present memoir. But most of the Halifax specimens are like those seen on Plate 24, figs. 22 and 27.

That fig. 20 is the *Kaloxylon Hookeri* of my former memoir is unquestionable. In that memoir I called attention to a special development of cells at the free extremity of each of the six radiating vascular wedges (*loc. cit.*, p. 15). The cells occupying the peripheral ends of the six primary medullary rays separating the six radiating vascular wedges were enclosed within a wavy line round, and at a little distance from, the end of each of those wedges, where "they enclose a small semi-lunar area (*g*) co-extensive with the diameter of the wedge, and which is occupied by a distinct form of cellular tissue. I shall shortly give my reasons for believing that this latter tissue is a quasi-cambial meristem layer, which is concerned in the formation of the newest exogenous vascular growth."

More extended investigations into the structure of this curious plant lead me to the conclusion that the above extract embodies a truth, but not all the truth. Plate 23, fig. 20, represents an excellent example of this stem from Halifax. As in the corresponding Oldham examples, the central axis is a bundle of vessels apparently unmixed with any cellular tissue. The radiating wedges, *c* (which are here five, not six, in number), and their five intervening primary medullary rays, *g*, do not differ from those of the Oldham forms. But the semi-lunar areas of the Oldham specimens represented in fig. 15, *g*, of the memoir quoted in the above paragraph are clearly seen in fig. 20, *k, k'*, of this memoir, to be the true phloem-masses of the several radiating wedges, and not a quasi-cambium as previously surmised; but which must, when the plant was living, have had a true cambium separating the inner border of each phloem from the periphery of the xylem. Plate 23, fig. 21, represents the phloem of the radial bundle, *k*, of fig. 20, enlarged 62 diameters; at *p'* we have the long narrow cells of the innermost margin of the cortex pursuing their undulating course, sweeping outwards opposite the ends of the xylem-wedges to make room for the phloem-masses, *k, k'*, and curving inwards when crossing the outer ends of the primary medullary rays, *g, g*, as at *g', g'*. There can be no doubt, therefore, that the *Kaloxylon Hookeri* was a true exogen, having a perfect cambium, which developed both xylem and phloem, but the traces of which cambium are but very imperfectly, if at all, preserved.

Plate 24, fig. 22, is a form from Halifax, in which the central axial bundle is in process of development, but in which no exogenous growth has yet taken place. Its thick cortical layer, *r*, from its hypodermal portion to within a short distance from the vascular bundle, has been very uniform in its composition. This structure was, as I have already observed, imperfectly preserved in the Oldham specimens, but soon after my description of the latter was published I obtained from Halifax several of those now described. The parenchyma of this cortex has been thin-walled, and the forms of its cells apparently rather irregular; but scattered amongst the latter are numerous long, narrow, longitudinally arranged canals, *r'*. It is not quite easy to determine whether these canals are true tubes or merely intercellular passages. Plate 23, figs. 24 and 25, demonstrate that each canal is enclosed by a wall, *r'*, within which we constantly find a black substance, evidently moulded upon the interior of this wall, either in the form of a solid rod, as in fig. 24, *r''*, or as a hollow cylinder, as in fig. 25, *r'''*. The wall, *r'*, may either be a true one belonging to the canal, or it may only consist of the coalesced walls of the surrounding cells. Seeing how long different opinions prevailed respecting the structure of such organs as the so-called laticiferous vessels, a determination of such a point in the case of fossil plants may well be doubtful. That these passages have been gum or resin canals may be inferred from the large amount of the black carbon which they so often contain. Fig. 26 is a section of a specimen from Halifax, in which five vascular wedges have undergone a considerable development, though much less than fig. 20. The inner ends, *d'*, *d'*, of the vascular laminæ composing each wedge curve away from its centre, converging towards similar laminæ belonging to contiguous wedges to form a series of semicircular curves. I further discover in similar specimens a few parenchymatous cells creeping in amongst the vessels, *e*, of the central axis. Such examples constitute connecting links between the type represented in fig. 20 and others yet to be described. The phloem masses are again visible at *k*, *k*. The epidermal layer, *p''*, so extremely characteristic of this plant, is here thicker than usual, consisting of from three to four cells in breadth, the ordinary number being two or occasionally three.

The specimen just described conducts us to fig. 27, which is an excellent example of the type most frequently obtained from the Halifax deposits. Its medullary axis, which is of large size compared with that of fig. 20, has an undulating peripheral outline of a pentagonal or hexagonal form; some specimens having the former, others the latter contour. The conspicuous bundles of vessels, *e*, are now largely intermingled with a delicate parenchyma, *a*. We find here no exogenous growths, unless the single continuous line of vessels, *e'*, seen bounding each peripheral concavity with more or less regularity, is to be regarded as the first of a series of such growths. This vasculo-cellular axis is completely invested by a zone of extremely delicate parenchyma, *p*, the innermost portions of which, especially where they fill the concavities, *p'*, of the peripheral outline, consist of very minute cells. Other specimens, resembling fig. 27 in most of their features, exhibit a wavy line of cortical cells,

indicative of the beginnings of exogenous development by the formation of phloem elements like those of fig. 26, *k*.

The specimen before us possesses additional interest from displaying a longitudinal section of what appears to be a rootlet, *y*, springing from the periphery of the medullary axis at *y'*, and pursuing its outward way through the bark. We see no vessels in this rootlet, since the section has only passed through the cellular zones forming its cortical cylinder; but another similar specimen in my cabinet exhibits such vessels in their normal central position.

I may now call attention to a remarkable series of objects, chiefly from Oldham, which are certainly organs of the plant under consideration. They vary in relative size from that represented at Plate 24, fig. 28, to fig. 37. So far as their general features are concerned, these objects differ but little from each other. In all we have the epidermal layer *p''* enclosing the cortex *r*, in the centre of which latter is a vascular bundle *e*. The chief interest of these specimens resides in the illustration they afford of the gradual development of this vascular bundle, and the conclusions suggested by that development as to the nature of the objects to which the bundles belong. Being from Oldham, the cortex *r* of each of these specimens is in the same imperfect state of preservation as characterised those figured in my Memoir, Part VII.; but, as the vascular bundle is now the object under consideration, this defect is of no present importance.

In Plate 24, fig. 28, we find the section of the bundle *e* of an oblong form. There are faint traces of small vessels given off from each side of the centre of this bundle, but they are too indistinctly preserved to be relied upon. In Plate 23, fig. 29, we again discover the bundle at *e*, and at Plate 24, fig. 29A, this bundle is enlarged 182 diameters. Most of the structures in this latter figure are certainly vessels or tracheids. A few of those marked *p*, *p*, may possibly be inner cortical cells. We here see the section of the bundle approaching a triangular, if not a quadrangular, contour. In Plate 24, fig. 30, *c*, we have a fine bundle from a Halifax specimen, enlarged 156 diameters, enclosed within a mass of small cells, *p*, which in turn are surrounded by the ordinary cortical parenchyma, *p'*. In this bundle we have a striking resemblance to that of a tetrarch rootlet which had developed centripetally; the vessels at the four primitive points of apparent protoxylem being very small, whilst the central ones, assumably of later growth, are of much larger size. Such was long my interpretation of this and similar specimens, but we shall see that the further study of other examples throws serious doubt upon the accuracy of this determination. Plate 24, figs. 31 and 32, represents transverse sections of two other bundles, the former enlarged 77 and the latter 99 diameters. Both these bundles, which are from Halifax specimens, present the same quadrate form, suggestive of a tetrarch origin, as fig. 30, and each bundle is imbedded in an investment of extremely delicate parenchyma, obviously identical with that (*p'*) surrounding the vasculo-cellular axis of Plate 24, fig. 27.

Plate 24, fig. 33, is a still smaller specimen of the type of fig. 28, from Oldham,

enlarged 39 diameters; its vascular bundle is shown, enlarged 187 diameters, in fig. 33A. This example closely resembles a triarch rootlet-bundle. Plate 24, fig. 34, is yet smaller, also from Oldham, and enlarged 39 diameters. Its bundle, *e*, enlarged 187 diameters, is represented in fig. 34A. It now consists of but six vessels. Figs. 35, 36, and 37 are three sections of these root-like structures from Oldham, becoming successively smaller, so that in fig. 37 the epidermal layer, *p''*, now consists only of a single layer of cells enclosing but a very small number of cortical ones. The vascular bundle of fig. 35, seen at fig. 35A, is now distinguished with difficulty from the cells by which it is surrounded. So far as I can determine, it consists of the five vessels marked *e*, *e*. In like manner, the bundle of fig. 36, represented at fig. 36A, seems to consist of the three vessels marked *e*, *e*; whilst that of fig. 37, shown at fig. 37A, seems to consist of the two vessels indicated by the same letters. All the Oldham specimens just described, viz., figs. 28, 29, 33, 34, 35, 36, and 37, belong to one great cluster of root-like examples crowded together in one slide; amongst them are other, more completely developed specimens, showing that the entire series belongs to *Kaloxylon Hookeri*. My first impression was that the whole cluster consisted of a series of rootlets, and the study of such examples as figs. 30, 31, and 32 not only seemed to confirm this opinion, but to show that they were rootlets with a tetrarch development of their several primary xylem bundles. But on tracing the development of those bundles downwards through such examples as figs. 34, 35, 36, 37, it became evident that, if the objects of which they formed a part were rootlets, the bundles had not been developed centripetally in the way characteristic of living rootlet-bundles, and which also was the case with other plants (*e.g.*, *Stigmaria Ficoides*) that lived during the Carboniferous age. Nevertheless, it is difficult to believe that these organs have been other than roots; though the apparently centrifugal growth of their bundles is more suggestive of an axial cauline development than of radicular structures.*

We may now ask: Can any light be thrown upon the systematic affinities of the two plants, *Heterangium Tiliacoides* and *Kaloxylon Hookeri*, described in the preceding

* [I find amongst the Carboniferous plants other examples of what appear to be branching stems, which diminish gradually in diameter, until, as in the instance described in the text, they become extremely slender. At the same time that they are reduced in size, they rapidly increase in the number of the transverse sections of them that are met with in our slides. The *Rachiopteris Oldhamium*, described in my Memoir, Part VI. ('Phil. Trans.,' Part 2, 1874), presents these conditions. The figures 20-24 on Plate 53 of that memoir show a gradation of diameters of sections from about .125 to .006 of an inch; yet none of these sections display the slightest trace of any foliar appendages. Stems thus gradually diminishing in diameter, whilst their numbers are multiplied, must be reduced either to the condition of aerial twigs or of subdividing roots and rootlets. If the former, where are their foliar appendages? If the latter, why do not their fibro-vascular bundles show the symmetrical arrangement telling of the centripetal development so characteristic of the proto-xylems of all roots? Of course the idea suggests itself that these curious objects may have had some relationships with the rootless subterranean branches of *Psilotum*, though many difficulties interfere with our ready acceptance of this explanation.—August 12th, 1887.]

pages? One fact is undeniable, viz. : that certain portions of them, obviously stems or branches, possessed, when living, a true cambium which developed xylem and phloem in a normally exogenous manner. The same statement applies to two other plants described in previous memoirs, and which must be considered along with those just named. These are *Lyginodendron Oldhamium* and *Heterangium Grievii*; besides which close affinities appear to exist between the *Lyginodendron* and *Rachiopteris aspera*, also described in the Memoir, Part VI.

Canals, precisely like those in the bark of *Kaloxylon Hookeri*, exist equally in the inner bark of *Lyginodendron* and of *Rachiopteris aspera*. The latter is unquestionably a rachis of a Fern; whilst the former displays a wonderfully clear exogenous development of its xylem zone. I have more than once, in previous memoirs, suggested that the *R. aspera* was the petiole of the leaf of the *Lyginodendron*; and my friend, the Count DE SOLMS, of Gottenburg, who has obtained numerous examples of these two plants from the Westphalian deposit at Pith Vollmond, has arrived independently at the same conclusion. If we are correct in this supposition, we have now, for the first time, in *Lyginodendron Oldhamium*, a Fern of which the stem or rachis exhibits a highly developed form of exogenous growth. This fact in some degree influences our interpretations of the two species of *Heterangium*, in both of which we find in the outermost bark the remarkable horizontal bands of dense sclerenchyma represented in Plate 22, fig. 11, of the present memoir, and in Plate 29, fig. 32, *h'*, and Plate 31, figs. 45, 47, and 49 of the Memoir, Part IV., in which *Heterangium Grievii* was first described. This remarkable peculiarity in the structure of the outer cortex of these two plants has led to a careful search for any fossil stems, with their foliage attached, in which a similar structure seemed to exist. Some months ago Mr. KIDSON sent me some stems which he believed to belong to *Sphenopteris elegans*, the cortex of which displayed an exactly similar series of thickened horizontal parallel bands. Still more recently he received from my friend Professor VON WEISS, of Berlin, and forwarded to me, a beautiful specimen of an exactly identical stem, attached to which are the unquestionable pinnules of *Sphenopteris elegans*. So far as these internally structureless specimens affect the question, they suggest the possibility that both the species of *Heterangium* may also prove to be Ferns. At first sight, remembering the exogenous growth developing both xylem and phloem, as well as the discigerous tracheids of *Heterangium Tiliæoides*, this idea seems to be a most improbable one; but it is no less probable than that *Lyginodendron Oldhamium* belongs to the group of Ferns, which latter conclusion has now made a near approach to certainty.

The extraordinary vasculo-cellular structure of the medullary axis of the two *Heterangiums* finds its parallel in such examples of *Kaloxylon Hookeri* as that represented in Plate 24, fig. 27, of the present memoir. To see anything approaching this structure in other plants, we must go back to the somewhat similar central axis of *Lepidodendron Selaginoides* (Memoir, Part XI, figs. 3, 5, and 10), which presents

equally anomalous combinations of internal structure, that remove it far from any known living form of vegetation, though it is an unquestionable Lycopod.

We thus fail, for the present, to reach anything beyond probabilities respecting the true affinities of the plants described in this memoir; but, whatever may be finally determined as to their real systematic position, one thing is certain, viz., that in their internal organisation they present combinations of tissues that find no representatives amongst living plants. Possibly they are the generalised ancestors of both Ferns and Cycads, which transmitted their external contours to the former and their exogenous modes of growth to the latter types. In considering this possibility, we must not forget that in *Stangeria* we have a still living plant in which the stem of a Cycad bears fronds, the leaflets of which retain the dichotomous nervation of a true Fern. The *Stangeria* has retained, not only the primitive exogenous stem of some ancestral type, in common with its other Cycadean relatives, but also the peculiar Fern-like leaflets, which may also have come down to it from Palæozoic times. Hence we have here a combination of Fern-like features and of an exogenous mode of growth. Such being the case, it need not startle us if we have to conclude that a similar combination existed during the Carboniferous age.

In closing this memoir, I have again to thank Mr. SPENCER and Mr. BINNS, of Halifax, for the specimens with which they have so kindly supplied me. But my thanks are most specially due to WILLIAM CASH, Esq. Resident on the spot, his incessant vigilance in seeking for new material and opening out new sources of supply has a value to me of which I can scarcely speak too highly.

INDEX TO THE PLATES.

Letters of reference to the figures of Heterangium Tilixoides.

Plates 21 and 22, figs. 1-19.

- A, Vasculo-cellular medullary axis.
 - a, Medullary cells.
 - b, „ vessels.
- B, Exogenous xylem.
 - c, Vascular wedges or bundles.
 - d, „ laminæ.
 - e, f, Vessels or tracheids.*
 - g, Primary medullary rays.
 - h, Secondary „ „
 - i, Cambial line.
- C, Phloem zone.
 - k, Phloem of the vascular bundles.
 - l, „ tubes.
 - m, „ parenchyma.
 - n, Primary phloem rays.
 - o, Secondary phloem rays.
- D, Cortex.
 - p, Inner cortex.
 - r, Outer „
 - s, Cortical cells.
 - t, Cortical bands of sclerenchyma.
 - u, Double vascular bundles.
 - w, Lateral branch.
 - x, „ „

* I have employed these two terms throughout the memoir without attempting to determine which of them may be applicable to the objects described. It is impossible to distinguish vessels from tracheids in these Carboniferous fossil plants.

| Figure. | Plate. | <i>Heterangium Tiliroides.</i> |
|---------|--------|--|
| 1 | 21 | Transverse section of a stem. $\times 15$ diameters. Cabinet number 1302, pp. 289, 290, 291. |
| 2 | 22 | Portion of a transverse section of another specimen. $\times 12$ diameters. C. N. 1303, pp. 290, 291. |
| 3 | 21 | Portion of a transverse section of a medullary axis. $\times 103$ diameters. C. N. 1301, p. 289. |
| 4 | 21 | Portion of a transverse section showing the phloem of a vascular wedge or bundle, and parts of two phloem rays. $\times 48$. C. N. 1303, p. 290. |
| 5 | 22 | Part of a transverse section with two wedges or vascular bundles being pushed outwards for some unknown purpose. $\times 24$. C. N. 1303, pp. 290, 293. |
| 6 | 21 | Part of a transverse section of the outermost cortex. $\times 31$. C. N. 1303, pp. 290, 291. |
| 7 | 22 | Transverse section of a pair of the twin vascular bundles passing outwards through the outer bark. $\times 51\frac{1}{2}$. C. N. 1623, p. 290. |
| 8 | 22 | Two other twin bundles. $\times 20$ diameters. C. N. 1302, pp. 290, 291. |
| 9 | 23 | Obliquely longitudinal section through another stem, showing the tissues of the medullary axis, the exogenous xylem, phloem, and part of the inner cortex. $\times 36$ diameters. C. N. 1628, p. 291. |
| 10 | 21 | Tangential section through the phloem of a fibro-vascular bundle. $\times 14$ diameters. C. N. 1622, p. 291. |
| 11 | 22 | Vertical radial section through the external cortex, showing three of the horizontal bands of sclerenchyma. $\times 31$ diameters. C. N. 1304, p. 292. |
| 12 | 23 | Vertical section through a stem, giving off a lateral branch, which is intersected in an obliquely transverse manner. C. N. 1622, p. 292. |
| 13 | 22 | Transverse section through one primary phloem ray and portions of two vascular wedges, each of which latter is giving off a cluster of tracheids to supply the vascular elements of a lateral branch. $\times 51$ diameters. C. N. 1619, p. 292. |
| 14 | 22 | A spiral vessel from a small bundle passing outwards to some lateral organ. $\times 112$ diameters. C. N. 1301, p. 293. |
| 15 | 22 | A reticulated vessel from the exogenous xylem of a stem. $\times 112$ diameters. C. N. 1621, p. 293. |
| 16 | 21 | A vessel with bordered pits from the exogenous zone of a stem. $\times 187$ diameters. C. N. 1621, p. 293. |

| Figure. | Plate. | <i>Heterangium Tiliæoides.</i> |
|---------|--------|--|
| 17 | 22 | A vessel only showing the canals of the bordered pits. $\times 112$ diameters. C. N. 1621, p. 293. |
| 18 | 22 | A vessel the lower part of which exhibits the bordered pits as in fig. 16, whilst in the upper part the canals alone are seen. $\times 184$ diameters. C. N. 1622, p. 293. |
| 19 | 21 | Cluster of cells from the cortex having the appearance of sclerous ones. Due probably to mineralisation. Page 293. |

Letters of reference to the figures of Kaloxylon Hookeri.

Plates 23 and 24, figs. 20–37A.

A, Medullary axis.

α , Medullary cells.

b , Vessels of medullary axis.

B, Exogenous xylem.

c , Vascular wedges.

d , „ laminæ.

e , Vessels and axial bundles.

g , Primary medullary rays.

h , Secondary „ „

i , Cambial line.

k , Phloem.

p, p' , Innermost cortex.

p'' , Epidermis.

r , Outer cortex.

r' , Canals of outer cortex.

y , Rootlet (?)

| Figure. | Plate. | <i>Kalorylon Hookeri.</i> |
|---------|--------|---|
| 20 | 23 | Transverse section of a stem with its axial vessels, exogenous wedges, and phloem, from Halifax. $\times 32$ diameters. C. N. 1238, p. 294. |
| 21 | 23 | Transverse section of the peripheral end of a vascular wedge of fig. 20, with its phloem enclosed within the wavy outline of the endoderm or inner border of the cortex. $\times 63$ diameters. C. N. 1238, p. 294. |
| 22 | 24 | Transverse section of a specimen from Halifax devoid of any exogenous zone, but with numerous tubular lacunæ in its cortex. $\times 51$ diameters. C. N. 1234, p. 295. |
| 23 | .. | By accident no corresponding figure. |
| 24 | 23 | One of the lacunæ of the cortex further enlarged, and with a solid carbonaceous substance in its interior. Page 295. |
| 25 | 23 | A similar lacuna to fig. 24, only with the carbon in its interior in the form of a ring. Page 295. |
| 26 | 23 | A transverse section from Halifax, the centre of which approximates to that of fig. 22, but which centre is surrounded by exogenous bundles with phloem elements. C. N. 1233, p. 295. |
| 27 | 24 | A fine transverse section from Halifax, with a considerable development of parenchyma in its vasculo-medullary axis, from the periphery of which a rootlet is being given off. $\times 51$ diameters. C. N. 1629, p. 295. |
| 28 | 24 | Transverse section of a rootlet (?) from Oldham. $\times 39$ diameters. C. N. 1239, p. 296. |
| 29 | 23 | A second, rather smaller, section like fig. 28. $\times 39$. C. N. 1239, p. 296. |
| 29A | 23 | Transverse section of the vascular bundle of fig. 29. Enlarged 187 diameters. Page 296. |
| 30 | 24 | Centre of a section from Halifax, with a quadrangular vascular bundle surrounded by a cylinder of small cells. $\times 156$ diameters. C. N. 1629, p. 296. |
| 31 | 24 | Obliquely transverse section of a vascular bundle of a specimen resembling fig. 30, with a quadrangular bundle surrounded by a delicate parenchyma. $\times 77$. C. N. 1234, p. 296. |
| 32 | 24 | A transverse section of a second example like fig. 31, from Halifax. $\times 99$. C. N. 1629, p. 296. |
| 33 | 24 | A transverse section of a small rootlet (?) from Oldham. $\times 39$ diameters. C. N. 1239, p. 296. |

| Figure. | Plate. | <i>Kaloxylon Hookeri.</i> |
|---------|--------|---|
| 33A | 24 | The vascular bundle of fig. 33. $\times 187$ diameters. Page 297. |
| 34 | 24 | Another section, yet smaller than fig. 33, from Oldham. $\times 39$. C. N. 1239, p. 297. |
| 34A | 24 | Vascular bundle of fig. 34. $\times 187$ diameters. |
| 35 | 24 | Transverse section of a still smaller rootlet from Oldham. $\times 39$ diameters. C. N. 1239, p. 297. |
| 35A | 24 | Vascular bundle and some attached cells of fig. 35. $\times 187$ diameters. C. N. 1239, p. 297. |
| 36 | 24 | Transverse section of a still smaller rootlet (?) from Oldham. $\times 39$ diameters. C. N. 1239, p. 297. |
| 36A | 24 | Vascular axis and some surrounding cells of fig. 36. $\times 187$. Page 297. |
| 37 | 24 | Transverse sections of one of the smallest rootlets (?) met with at Oldham. $\times 39$ diameters. C. N. 1239, p. 297. |
| 37A | 24 | The vascular axis and some attached cells of fig. 37. $\times 187$. Page 297. |

XIII. On *Gusterolichenes*: a New Type of the group *Lichenes*.

By GEORGE MASSEE.

Communicated by W. T. THISELTON DYER, M.A., C.M.G., F.R.S., Director, Royal Gardens, Kew.

Received May 5,—Read June 16, 1887.

[PLATE 25.]

UNTIL recently the time-honoured division of Thallogens into three primary groups, *Algæ*, *Fungi*, and *Lichenes*, was retained, the most prominent morphological features of the last group consisting in the vegetative portion being composed of slender achlorophyllose threads mixed with chlorophyll-producing cells or gonidia, and the spores produced in asci. It was assumed that the gonidia were in organic continuity with the colourless threads, an assumption challenged by SCHWENDENER,* who considers a lichen as a combination of a fungus and an alga, an idea now widely entertained, nevertheless, curiously enough, numbering amongst its opponents most of the leading lichenologists and mycologists of the day, most of whom, it is important to remember, are distinguished as systematists rather than as biologists. Later researches have placed almost beyond doubt the truth of SCHWENDENER's theory. BORNERT† has shown that in numerous instances the gonidia can with certainty be referred to some species of *Algæ*, and further succeeded in building up a lichen synthetically by sowing the spores of *Parmelia parietina* upon *Protococcus*. STAHL‡ also, by growing the spores and gonidia (*pleurococcus*) of *Endocarpon pusillum*, produced the lichen which bore perithecia and spermogonia. The same author has also demonstrated the presence of sexual organs of reproduction in a gelatinous lichen, *Collema microphyllum*, the female portion resembling the carpogonium, with its trichogyne, met with in the *Florideæ*. Spermatia, produced in the spermogonia, are the fertilising elements.

The discovery by MATTIROLI§ of a second type of lichen structure, *Hymenolichenes*, characterised by having the spores borne on basidia, the latter compacted into a

* "Untersuchungen über den Flechtenthallus," in Nägeli's 'Beiträge zur Wissensch. Botanik,' 1860, 1862, and 1868. See also 'Flora,' vol. 55, 1872 (Nos. 11-15).

† "Recherches sur les Gonidies des Lichens," 'Ann. Sci. Nat. (Bot.),' vol. 17, 1873.

‡ 'Beitr. zur Entwickel.-Gesch. der Flechten,' 1877.

§ "Contribuzioni allo Studio del genere *Cura*, Fr.," 'Nuovo Giorn. Botan. Ital.,' vol. 13, 1881, p. 245. (2 plates.) See also "Die Gruppe der Hymenolichenen," FRIED. JONOW., 'Pringsheim, Jahrb. Botan.,' vol. 15, p. 361. (5 plates.)

continuous hymenium as in Hymenomycetous fungi, has also considerably strengthened SCHWENDENER's theory, as the mutual relations between the two components can be more readily determined than in the older group of *Ascolichenes*.

It is somewhat remarkable that a re-arrangement of Thallogens was not attempted earlier, considering the difficulties experienced by cryptogamists of half-a-century ago in locating the genera now included under *Hymenolichenes* and *Gasterolichenes*. *Dictyonema* was first described as an alga by C. A. AGARDH,* who placed it in the *Confervoideæ* next to *Hydrodictyon*, yet he appears to have been uncertain about its true position, owing to the numerous colourless anastomosing threads, and concludes with the remark, "Forsan Lichenis species!" NEES afterwards removed the genus to Fungi, changing the name to *Dichonema*, but was in turn quite as much perplexed on account of the numerous chlorophyll-bearing cells, and at the end of the generic description says, "Forsan thallus Cœnogonii." The same remarks apply to the genus *Cora*, described by FRIES† as a fungus, stating that at one time he considered it as belonging to Lichenes. In like manner, the Rev. M. J. BERKELEY, in the description of *Emericella*, clearly recognised the lichenose structure, suggested by the presence of green cells along with colourless threads, and also called special attention to the absolute agreement between the green cells and the *Palmella* previously described as an alga in the same work, finally pointing out that it differed from lichens in the mode of spore-formation. It is remarkable how nearly SCHWENDENER's views were anticipated in this instance by BERKELEY.

The genera already mentioned, along with *Laudatea*, JOH., *Rhipidonema*, MATT., *Emericella*, BERK., and *Trichocoma*, JUNGH., although universally acknowledged as Thallogens, are equally rejected by those lichenologists and mycologists opposed to SCHWENDENER's views; their peculiar non-ascigerous fructification alienating them from Lichens, and the chlorophyllose cells from Fungi, while algologists appear unable to reconcile themselves to the idea of an alga producing such fungal-like organs of fructification on the colourless hyphæ.

The object of the present communication is to describe a third type of lichen structure, resulting from the consortism of Fungi belonging to the *Gasteromycetes*, order *Trichogastres*, with unicellular algæ. Following the rule suggested by MATTIROLI,‡ the present section will take the name of *Gasterolichenes*.

The type *Emericella varicolor*, BERK., is described by the Rev. M. J. BERKELEY§ as follows:—"On the confines of *Myxogastres* we have the little group consisting of *Coniocybe*, *Byssophyton*, and a new genus, to which I have given the name of *Emericella*. These are in habit more or less Lichenose, but differ from *Calicium* and allied genera in the total absence of asci. *Emericella*, of which a figure is subjoined, consists of

* 'Syst. Alg.' p. 85.

† H. FRIES, 'Pl. homon.' p. 303.

‡ "Contribuzioni allo Studio del genere *Cora*, Fr.," 'Nuovo Giorn. Botan. Ital.,' vol. 13, 1881, p. 245. (2 plates.)

§ 'Intr. Crypt. Bot.' p. 340. (Cum ic.)

little oblong or clavate masses, varying in colour from yellow to green or grey. A vertical section shows a little peridium above, filled with threads and globose purplish spores, remarkable for a border of long spines, all situated in the same plane. The peridium is supported by a spongy central column, giving off threads which are terminated by large globose bodies resembling closely the gonidia of Lichens, but growing very much like the *Palmella** figured at p. 118. Dr. MONTAGNE has observed these bodies to become blue with iodine, but this is not confirmed by myself or Mr. BROOME. I have, in fact, tried various preparations of iodine, and the addition of sulphuric acid has given no blue tinge. The general colour of the plant does not arise from these bodies so much as from the fine threads on which they grow. Increase in many cases certainly takes place exactly as in the *Palmella*, by the division of the central nucleus, and in one instance I have observed two of them to be confluent. This very curious fungus was gathered by my son, Lieutenant EMERIC SREATFIELD BERKELEY, in his garden at Bowenpilly, near Secunderabad. I have named it *Emericella varicolor*, and it is certainly one of the most curious that has ever come under my notice."

The plant is gregarious in habit and resembles in external appearance such Fungi as *Diderma* or *Tubulina*, and, although stated by BERKELEY to be on the confines of *Myxogastres*, is arranged by that author under *Trichogastres*. The shape varies from cylindrical to turbinate, measuring from two to three lines high by two lines in diameter. In structure, the genus approaches *Lycoperdon*, with which it agrees in the single membranaceous peridium and well-developed sterile base. The hyphæ of the sterile stem-like base are thick-walled, about 8μ in diameter, and form an interlaced central column surrounded by a loose peripheral plexus of branched hyphæ, which pass uninterruptedly upwards and form the peridium. The dense capillitium is continuous with the sterile base, and consists of branched tapering hyphæ of a pale yellow colour when viewed by transmitted light. The spores are purple-brown in the mass, globose, and furnished with a row of spines, all situated in one plane; diameter, including the spines, about 12μ . In *Trichogastres* the hyphæ concerned with reproduction have usually very thin walls, and disappear soon after the formation of the spores; consequently the mode of attachment of the spores could not be ascertained. Dehiscence takes place by the disintegration of the upper portion of the peridium, as in *Lycoperdon calatum*, the persistent capillitium remaining attached to the more or less marginate stem-like base. The alga is *Palmella botryoides*, GREV., which appears to differ in its larger size, and in being connected by green threads, from the plant described by RABENHORST† under the same name. The cells are sub-globose or broadly elliptical, varying from 20μ to 39μ in longest diameter, and furnished with a very thick, hyaline, lamellose cell-wall. From the chlorophyllose portion of the cell a green eseptate filament passes through the cell-wall, where it is joined at some distance to a

* The *Palmella* alluded to is *P. botryoides*, GREV.

† 'Flor. Eur. Alg.', vol. 3, p. 38.

similar thread from another cell, the two forming a common stem, on which several pairs of cells are supported on similar lateral bifurcating threads. These pairs of cells originate from the fission of a single cell, each half of the parent cell giving origin to a green filament, the bifurcation of which is at first included in the cell-wall. The alga occupies interspaces in the loose peripheral portion of the base of the fungus, and also passes up into the loose texture of the peridium, giving the yellowish-green tint to every external part of the plant before dehiscence. The tips of lateral branches of hyphæ are frequently seen closely investing and even penetrating the algal cells. *Trichocoma paradoxa*, JUNGH., is the type of a second genus of *Gasterolichenes*. This plant was first discovered by JUNGHUHN in Java, and was described as a fungus,* its very anomalous structure causing the author much uncertainty in referring it to any of the known divisions of Fungi. Finally it was placed in the *Hyphomycetes*, on account of some resemblance to such compact forms as *Stilbum*. MONTAGNE† afterwards considered it as having more affinity with the *Gasteromycetes*, in which family it has up to the present remained.

In habit the plant is gregarious, growing horizontally on decayed trunks or branches, in shape more or less cylindrical, and varying from three quarters to an inch and a half in height, by half an inch or more in diameter. The sterile basal portion is cup-shaped, and consists of thick-walled, eseptate, much-branched hyphæ, compacted into a dense pseudo-parenchymatous tissue. From the margin of this cup the hyphæ pass upwards and form a loose membranaceous peridium. The capillitium arises from the sterile basal portion, and consists of erect branched threads tapering upwards and compacted into a cylindrical tuft, which after the disappearance of the evanescent peridium resembles a camel's-hair brush springing from the cup-like base. In young specimens traces of the reproductive hyphæ may sometimes be met with, bearing basidia and sterigmata, proving the spores to be true basidiospores, as in *Lycoperdon*. The spores are brown, tinged with purple, in the mass, elliptical and coarsely warted, measuring about $6\mu \times 3\mu$. The alga belongs to KUTZING's genus *Botryococcus*, and forms a stratum at the base of the capillitium. In the dry plant this layer is bright-yellow, but the alga becomes green when moistened, especially if a small quantity of potassic hydrate is added to the water. The colonies vary in size, measuring on an average 25μ , and are generally invested with hyphæ, which in the "gonidial layer" assume a yellow tinge.

In addition to the locality given above, this species is represented in the Kew Herbarium from Sikkim, East Nepaul, Nilgiris, and Ceylon.

A smaller plant, included with *T. paradoxa* in the Kew Herbarium collection, proves on examination to be a new species, characterised as follows:—

Trichocoma levispora. Receptaculum basilare rotundato-cupulatum. Flocci elongati, comosi, in capitulum cylindricum persistens collecti, sporidiis subglobosis, lævibus,

* 'Verhand. van het Batav. Genootsch. van Kunsten en Wetensch.,' Batav., 1839.

† "Cryptogames de Java," 'Ann. Sci. Nat. (Bot.),' vol. 16, 1841, p. 308.

$4\mu \times 3\mu$. From half to two-thirds of an inch high. On rotten wood, Lower Carolina; resembling *T. paradoxa* in general appearance and structure, but differing in its smaller size and sub-globose smooth spores.

The group Lichenes can be divided into two natural sections, characterised by the nature of the fruit, which agrees with that produced by the two sections of Fungi, *Ascomycetes* and *Basidiomycetes* respectively. Each section can be further divided into two sub-sections, depending upon the exposed or concealed hymenium.

A.—*Ascolichenes*. Sporidia produced in asci.

I. *Discolichenes*. Hymenial surface exposed, as in *Discomycetes*.

II. *Pyrenolichenes*. Hymenial surface concealed in a perithecium, as in *Pyrenomycetes*.

B.—*Basidiolichenes*. Spores produced on basidia.

I. *Hymenolichenes*. Hymenial surface naked, as in *Hymenomycetes*.

II. *Gasterolichenes*. Hymenial surface concealed in a peridium, as in *Trichogastres*.

DESCRIPTION OF THE PLATE.

Fig. 1. *Emericella varicolor*, BERK., nat. size.

Fig. 2. The same in a young state, $\times 50$.

Fig. 3. The same after dehiscence, $\times 50$.

Fig. 4. Section of same before dehiscence, $\times 50$.

Fig. 5. Section of same after dehiscence, $\times 50$.

Fig. 6. Vertical section of same after dehiscence, showing the peripheral "gonidial layer," *a*, the central hyphal column, *b*, and the capillitium with its spores, *c*. $\times 300$.

Fig. 7. Portion of the alga *Palmella botryoides*, GREV. Cells of plant, *a*; supporting threads of same, *b*; hyphæ surrounding and entering the algal cells, *c* $\times 600$.

Fig. 8. Spores of same, $\times 600$.

Fig. 9. *Trichocoma paradoxa*, JUNGH., nat. size.

Figs. 10 and 11. Sections of same, nat. size.

Fig. 12. Section of same, showing sterile basal cup, *a*; "gonidial layer," *b*; and capillitium with spores, *c*. $\times 300$.

Fig. 13. Colonies of the alga, a species of *Botryococcus*, accompanied by hyphæ. $\times 600$.

Fig. 14. Spores of same, $\times 600$.

Fig. 14A. Basidium, with sterigmata of same, $\times 600$.

Fig. 15. *Trichocoma lavispora*, MASS. *a*, before rupture of peridium; *b*, after disappearance of peridium; nat. size.

Fig. 16. Spores of same, $\times 600$.

XIV. *An Enquiry into the Cause and Extent of a Special Colour-relation between certain exposed Lepidopterous Pupæ and the Surfaces which immediately surround them.*

By EDWARD B. POULTON, M.A., F.L.S.

Communicated by Professor E. RAY LANKESTER, F.R.S.

Received and Read, February 10. 1887.

[PLATE 26.]

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Introductory.

THE present investigation grew out of the enquiry into the colour-relation between Lepidopterous larvæ and their food-plants. In the search for the larval sense-organ which is affected by the light reflected from surrounding surfaces, great difficulty was experienced from the fact that the influences work slowly through the whole of larval life. Thus the ocelli must be eliminated by blinding five times in the life of each caterpillar, and there is always the chance of the skin being thrown off a little earlier than usual, in which case the success of the whole experiment might be endangered, for the coverings of the eyes are changed with the rest of the skin. It seemed probable that in the pupal colour-relation the influence must act during a short and rigidly limited time, in which experiments could be conducted with much hope of a successful isolation of the effective sense-organs.

Historical.

The first observation upon the colour-relation between Lepidopterous pupæ and surrounding surfaces was made by Mr. T. W. WOOD, who exhibited in 1867 a number of pupæ of *Papilio machaon*, *Pieris brassicae*, and *P. rapæ*, which corresponded in colour to the surfaces to which they were attached ('Entom. Soc. Proc.,' 1867, pp. xcix.-ci.). He also made some remarks on the subject, and expressed his opinion as to the nature of the susceptibility of these organisms in the following words:—"I find as the result of my experiments that the skin of the pupa is photographically sensitive for a few hours only after the caterpillar's skin has been shed; and, as might be expected, by putting the specimens in the sunshine at the time of changing, and surrounding them as much as possible with any desired colour, the most successful results have been obtained." There is no doubt that Mr. WOOD placed the larvæ under the conditions he describes, and that in consequence the pupal colours were influenced, but if he had transferred the larvæ immediately before pupation to a surface of an entirely different colour the pupæ would have corresponded with the earlier surroundings. It seems strange that it never occurred to any previous observer to test the theory of pupal susceptibility in this simple manner. Mr. WOOD exhibited green pupæ of *P. brassicae*, found under a vine on a stone-coloured house, while none of the pupæ on other parts of the same house were of this colour. Very dark pupæ were shown which had been taken from a tarred fence, and from other dark surfaces, and in subdued light. One of the pupæ of this species, found on a white surface, was nearly white, and Mr. WOOD also showed green, reddish, and dusky pupæ of *P. brassicae* on surfaces of similar tints. He also stated that the pupa of *Vanessa polychloros*, when amongst foliage, is coloured like a withered elm leaf, being light reddish brown with a cluster of silvery metallic spots (dorsally placed) at the junction of thorax and abdomen. He also rightly asserted that the gilded appearance is not necessarily connected with the presence of Ichneumon larvæ within the pupæ. When the pupa is suspended to a wall the gilded appearance is not produced, and the pupa is of a mottled greyish colour. This observation is very important, for it contained the obvious implication that the metallic appearance may be controlled by the surroundings, as I have now proved it to be. In fact, Mr. WOOD states, "I feel convinced that by the proper use of gilded surfaces the gilded chrysalides of *Vanessa*, and perhaps of other genera, would be obtained, and I hope to be able to try the experiment next season." I was not aware of Mr. WOOD's last suggestion in 1867 when I undertook my experiments on the subject in 1886, and the arguments which induced me to make use of gilded surfaces are given at the beginning of the experiments. It certainly does seem strange that so remarkable, although so bold, a suggestion should not have been subjected to any test for nearly twenty years. The reason is perhaps to be found in Mr. WOOD's unfortunate inclusion of *Papilio machaon* among the list of susceptible pupæ; for Mr. BOND, who knew the habits of this

species (which Mr. WOOD expressly affirms were unknown to him), at once stated in the discussion which followed the paper that "he had had thousands of pupæ of *Papilio machaon*, and had often had the brown variety of pupa on a green ground-colour, whilst in some seasons he had obtained no brown specimens at all." My own experiments also clearly show, as far as the numbers employed could do so, that this species has no trace of susceptibility to surrounding colours. Mr. A. G. BUTLER also stated in the discussion that "he had obtained a red or rosy chrysalis of *Pieris rapæ*, which had undergone its transformation in a piece of scarlet cloth; and pupæ upon glass were generally of a pale slate colour." Concerning the former observation, *P. rapæ* is very commonly tinged with pink, and I think it almost certain that if the pupa was not removed from the scarlet surroundings the reflected light would intensify the pink tinge of the ground colour, and would thus produce a simulated resemblance between the two. I found that it was never safe to compare the colours of pupæ until they had been removed from the coloured surface on which pupation had taken place, and were arranged side by side upon white paper. Furthermore, in my experiments a dark red surface did not produce reddish, but dark, pupæ in *P. brassicæ*, and (as will be shown below) I can quite confirm Mr. BOND's criticism that reddish pupæ of *P. rapæ* are not found on red brick walls. At the same time Mr. BOND's objection to the main position taken up by Mr. WOOD—that the pupæ of *Pieridæ* have some general resemblance with their surroundings—is without sufficient foundation, and the fact must now be generally admitted. His criticism that he has seen pupæ of *P. rapæ* with all shades of colour on the white painted woodwork of a greenhouse is valueless unless he can show that the dark forms are as common as when the pupæ are found on a tarred fence. Mr. BOND's observation that the variable pupæ of *Anthracis cardamines* are not sensitive is important, but the species needs experimental investigation in order to confirm his observations.

The late Mr. EDWARD NEWMAN also expresses an opinion adverse to Mr. WOOD's observations upon *P. rapæ* in these words ('British Butterflies,' 1871, p. 162), "An ingenious—but, as I think, futile—attempt has been made to show that the colour of the chrysalis varies with the colour of the object to which it is attached."

Furthermore, Rev. J. HELLINS ('Larvæ of British Butterflies,' &c., by WILLIAM BUCKLER (Ray Society), 1885, p. 155) states of the pupæ of *P. rapæ*, "The colour seems very varied, but, as all the varieties occurred side by side on the cover of the tin box in which my larvæ were reared, I could not account for their difference." And of the different forms of *P. brassicæ* he also says, "These varieties were developed side by side in the same cage." It will be abundantly proved below that only *certain* colours affect each species which is sensitive to surrounding colours, and when other colours are made use of the pupæ either assume the commonest form, or, if very variable, are free to obey the influence of their varied hereditary or individual tendencies. And the use of tin as a background for the *Pieridæ* is especially likely to produce such results, for this surface is extremely unlike any of their natural

surroundings. Furthermore, it will be shown that the colour-relation is due to larval susceptibility during very many hours before pupation, so that, unless special precautions are taken, the larvæ may be disturbed or removed from one surface to another in feeding, observing, &c., and the results are likely to be highly irregular, for the boxes would always contain one colour which acts powerfully upon these two species, *i.e.*, the green leaves of the food-plant.

Professor MELDOLA, in a paper communicated to the Zoological Society ("On a certain Class of Cases of variable Protective Colouring in Insects," 'Zool. Soc. Proc.,' 1873, p. 153) confirms Mr. T. W. WOOD's observations on the pupæ of the Pieridæ, for he states (p. 156), "I have observed a similar fact with respect to the pupæ of *Synchlora* (*Pieris*) *brassicæ* and *S. rapæ*, specimens from a black fence being generally darker than those found on walls." Professor MELDOLA informs me that this conclusion was obtained from the comparison of large numbers of individuals.

The next observation of the correspondence between the colours of variable pupæ and that of their surroundings is found in a paper by Mrs. M. E. BARBER, communicated by Mr. DARWIN to the Entomological Society of London ('Entom. Soc. Trans.,' 1874, p. 519). Mrs. BARBER had experimented with the pupæ of *Papilio nireus*, common in most parts of the Cape Colony. The larva itself in this species has also the power of colour adaptation to its surroundings, for Mrs. BARBER states that it is dark-green when found feeding upon orange trees, and lighter green upon *Vepreis lanceolatu*. In the natural state the pupal colours are always similar to those of the leaves of the food-plant, when pupation takes place among the leafy twigs—its usual surroundings. In Mrs. BARBER's experiment the larvæ were reared "in a case with a glass cover; the case was partly made of wood and partly of brick: the colour of the wood was a dullish yellow, that of the brick a purplish-brown." Orange leaves, on which the larvæ fed, were placed in the case, and also a branch of the common bottle-brush shrub, of which the dead leaves were pale-green. Of the resulting pupæ some were fixed to the orange leaves, and were of the usual deep-green colour, like that of the surrounding leaves; others were fixed "to the bottle-brush branch, and these became pale yellowish-green pupæ of precisely the same colour as the half-dried leaves. One of the caterpillars in particular affixed itself upon the wooden framework of the case, where the wood and the brick came in contact with each other, and to my surprise this caterpillar, after throwing off its bright-green skin, assumed the colours of *both the wood and the brick*, its under-side resembling that of the wood to which it was attached, and the upper side that of the adjacent brickwork."

"Some days later another specimen affixed itself to the wooden frame of the case, and then became a yellowish pupa of the same colour as the wooden frame." Mrs. BARBER then tried the effect of surrounding a caterpillar before pupation with scarlet cloth, but the resulting pupa was of the common deep-green form, in which, however, the coloured spots usually present in this variety were of a brighter red. Mrs. BARBER suggests that these correspondences of colour may be analogous to those

of the Chamæleon, or may be "a sun-picture or photograph"; and in support of the latter view (which has been commonly accepted up to the present date) the writer calls attention to the transparent surface of the freshly-formed pupa, which might be sensitive to light. In my experiments, described below, I did not meet with any parti-coloured pupæ, and it is to be noted that a difference between the colours of the dorsal and ventral surfaces is common in pupæ. It would be satisfactory to expose this highly sensitive insect to conflicting colours arranged antero-posteriorly instead of dorso-ventrally. Although Mrs. BARBER's figure certainly supports the conclusion that the different pupal surfaces may be influenced independently, the conclusion is too important to rest on a single instance, and it is to be much hoped that the subject will be re-investigated. In the discussion which followed Mrs. BARBER's paper ('Entom. Soc. Proc.,' 1874, p. xxiv.), Professor MELDOLA remarked that "the action of light upon the sensitive skin of a pupa had no analogy with its action on any known photographic chemical. No known substance retained permanently the colour reflected on it by adjacent objects."

Mr. J. P. MANSEL WEALE makes a further interesting addition to the history of the subject in his record of experiments upon the larvæ of *Acræa esebria* (see 'Entom. Soc. Trans.,' 1877, p. 271), also in South Africa. He says: "Some larvæ I confined in a dark box, and found that the coloration of the pupæ (usually white, with thin black and orange markings) was materially altered and darkened, so as closely to resemble those of *Acræa horta*, but the individuals were as varied in colour as those raised under ordinary daylight. This darkening of colour I have found to be very common under the same circumstances in pupæ belonging to different families of Butterflies. Both larvæ and pupæ of those *Acræas* known to me are found in conspicuous places without any attempt at concealment."

Mr. J. P. MANSEL WEALE also records, on p. 275 of the same paper, some interesting experiments upon the variable pupæ of *Callosune keiskamma* and those of *Eronia cleodora*. He says: "With reference to the changes in the colour of pupæ, I believe a very wide field of research is open, and with the improved modes of microscopical examination under the spectroscope important revelations on the subject of variation will be discovered. I here give the results of some very rude experiments on this subject. Most of the specimens were reared in glass test-tubes exposed on coloured cards, in which they were partially enveloped. They were constantly supplied with as little food as possible, in order that their full exposure to the colour should not be interfered with.

"Pupæ of *C. keiskamma*."

- | | |
|--|-----------------------|
| (1.) On dead leaves away from light | Dark brown. |
| (2.) On stem and on vermilion cards | Pale ochreous. |
| (3.) On vermilion card | Pale bluish-green. |
| (4.) Exposed on bush in nature and on yellow gamboge cards . . | Bright green. |
| (5.) On glass tumbler | Pale yellowish-green. |
| (6.) On green (cobalt and gamboge) | Ochreous. |
| (7.) On cobalt-blue | Greenish-white. |

"On a white surface pupæ of *Eronia cleodora* became so pale-coloured as to be almost translucent, the marks on the ventral aspect of the abdomen being almost obliterated, and the bright yellow-green colour usually predominant almost invisible."

Mr. TRIMEN confirmed Mrs. BARBER's observations upon *P. nireus* in some experiments conducted upon *Papilio demoleus*, which is common at Cape Town. These results have not been published, but Mr. TRIMEN has kindly sent me an account of them in a letter. He says, "I left my *Demoleus* larvæ full liberty, within the range of their breeding-cage, to attach themselves to any of the bands of colour with which the sides of the cage were provided. I had noticed in nature that the pupæ attached to the green upper twigs of the food-plants among the leaves were always more or less green or greenish, while those on the more brownish or greyish stems were coloured like the latter. Though more of these larvæ suspended themselves to the food-plant, the remainder showed no apparent choice among the colours, but attached themselves indiscriminately all about. The colours which seemed certainly to directly affect those of the pupæ were (irrespective of black, which made them a little darker) green, yellow, and reddish-brown, these tints being more or less reproduced. Bright red and bright blue had no apparent effect. I did not repeat the experiment, finding this one so confirmatory of Mrs. BARBER's observations on another species of the same genus."

Finally, Professor MELDOLA, knowing that I was looking out for any notices of the literature of the subject, kindly sent me the following translation of an important note in 'Kosmos,' by FRITZ MÜLLER, showing that the pupæ of *Papilio polydamus*, although dimorphic—green and brown—like our own *P. machaon*, are, nevertheless, like it, not susceptible to the influence of surrounding colours.

"According to the observations and experiments of WOOD on *P. rapæ*, and of Mrs. BARBER on *P. nireus*, the colour of the pupæ in these Butterflies is determined by the colour of the object on which they pupate. Nevertheless, this does not hold good for all Butterflies of which the pupæ are differently coloured; it is not the case, e.g., in *P. polydamus*. The pupæ of this Butterfly, which in former years I saw in large numbers, are either green or brown, and I have never found intermediate colours. The ground colour of the larva which lives on *Aristolochia* varies within wide limits; pure black and bright yellowish or reddish-brown caterpillars are seldom found, but all possible stages between these extreme colours are common. The colour of the larva, however, has nothing to do with that of the pupa, and from both kinds of pupæ similarly coloured Butterflies are developed, both males and females.

The Butterfly always lays several (4 to 6) eggs close together; till the second moult, the young larvæ also keep together; they eat the same leaf and sit close together when at rest (like the social larvæ of *P. evander*, till they pupate). Such a society of young larvæ that I observed from the eggs in my garden I recently transferred to a large glass case before they distributed themselves over different leaves, and from this, as they prepared for pupation (i.e., when their excrement,

instead of being hard and dry, became fluid), to a box, of which the two larger sides consisted of white gauze, and the narrower sides and the bottom and top (cover) of grey pasteboard. They fastened themselves to a thin defoliated stalk of *Aristolochia*. Of the five larvæ, two changed into brown and three into green pupæ; a brown and green pupa were on the same stalk, removed by less than their own length from each other. They emerged from the egg at the same time and shed their larval skin at the same time, whilst during their whole life (larval) they were exposed to the same external conditions, the same action of light, and to the time of pupation had neither brown nor green in their surroundings. In this case, therefore, the influence of the colour of the environment certainly cannot have affected the colour of the pupa." ('Kosmos,' vol. 12, p. 448.)

Previously accepted explanations.

The theory of the moist, fresh, pupal surface as "photographically sensitive" was obviously a metaphor borrowed from the sensitive plate of photography, and Professor MELDOLA pointed out that there could be no real analogy between the two processes. Furthermore, there was the difficulty that the explanation failed to account for the colour of those pupæ which throw off the larval skin on a dark night, for the pupal colours very quickly deepen into their permanent condition. Considering these difficulties, and knowing that the explanation had never been tested by "transference" experiments, I came to the investigation with the firm conviction that it would be found that the problem was essentially physiological, and that the physico-chemical changes were merely the results of far more complicated physiological processes. It furthermore seemed probable that the reflected light would be found to act—for a period long enough to include, under any circumstances, many hours of daylight—upon some sensitive area in the larva as it rested upon a coloured surface before pupation, and it appeared likely that such a sensitive area might be defined by experiment. The investigation was conducted during the summer and autumn of 1886. The object of the present paper is to give an account of the investigation of the questions alluded to above, and its results are therefore preliminary in the sense that they afford a foundation for future work, in which the physiological changes induced by varying colour must be sought out by histological and other methods. Such an investigation I hope shortly to undertake.

Experiments upon Vanessa Io.

This pupa is dimorphic, the common form being "pale grey, but freckled all over with smoky black" (BUCKLER), with a small amount of gilding, while the less common form is bright yellowish-green, with a large amount of gold. I determined to ascertain whether the latter form could be produced by placing the larvæ in

appropriate coloured surroundings before pupation. Mr. E. D. Y. PODE very kindly sent me six nearly full-grown larvæ from South Devonshire, and these were placed in a glass cylinder covered with two thicknesses of green tissue paper, of which the outer layer had become very yellowish from the action of light. The paper being very transparent, the larvæ in the cylinder were exposed to a yellowish-green light, mixed with a great deal of white light. All the six larvæ suspended themselves from the paper roof of the cylinder, and five changed into the yellowish-green variety. One of these was figured (see Plate 26, fig. 7; natural size). The sixth was detected a few minutes after the larval skin had been thrown off, for the surface was moist and the shape unformed. It was exactly in the condition of assumed photographic sensitiveness, described by previous observers. I therefore cut it down immediately and pinned it up in an opaque box with a tightly fitting lid, the whole interior surface being lined with black paper. In a few hours I opened the box, and found the pupa a yellowish-green variety, exactly like the others. It is therefore quite clear that the influence had worked previously—during the larval stage. There can be no doubt that this result without a single exception, is conclusive, when the comparative rarity of the green variety is considered. In all my previous experience I have only obtained this variety singly among large numbers of the dark form, and I have *never* seen it among the numerous pupæ found upon palings and walls, and I have never found the pupa of this species in other situations—upon the leaves of its food-plant or other plants. Being anxious to ascertain whether other observers have had the latter experience, and to know its results, I wrote to Mr. W. H. HARWOOD, of Colchester, who has been a most keen observer for many years. In his reply he says, “I have sometimes found the pale form of *Io* on the under-side of nettle leaves, but do not remember meeting with the dark one.” From this observation it is seen that the power of colour adaptation, which experiment has proved to exist in this species, is actually turned to account in the wild state. NEWMAN, in ‘British Butterflies,’ p. 61, does not recognise the pupal dimorphism, for he says, “the colour of the chrysalis is green; as the chrysalis darkens its colour deepens, but the green tint is never entirely lost.” BUCKLER, on the other hand (Ray Society, 1885), fully recognises the two forms. While the single individual, which was transplanted to a colour which must presumably tend towards an opposite effect, seems conclusive against the former theory of pupal as opposed to larval sensitiveness, the same results are better seen in *Vanessa urtica*, where they were worked out in great detail, and in which the proof becomes irresistible from the large numbers employed in the experiments. The cuticles of the left pupal wings of the two varieties are figured in Plate 26, figs. 10 and 11, both $\times 7$, and the immense differences in the tint of the cuticular ground colour and the amounts of pigment present are well seen.

Experiments upon Vanessa urticæ.

There are certain reasons why this species is peculiarly fitted for the purpose of the present investigation. It is exceedingly common throughout the whole of the summer months, and its food-plant—nettle—is abundant everywhere; above all, the larvæ are gregarious, living on webs when young, scattering when older, but never to such an extent that more than a few yards intervene between the most widely separated larvæ. Such companies are made up of large numbers of larvæ (examples of the numbers will be given below), and, as each of them results from the eggs laid by a single butterfly, it follows that in any series of experiments conducted upon the individuals of a company the possible errors which might follow from different hereditary tendencies due to different parents—of unknown, but presumably varying, histories in the larval state—are completely eliminated. At the same time, the numbers are amply sufficient to admit of the experimental investigation being varied and, at the same time, carried on in the most complete manner among the offspring of a single pair of butterflies. During the whole summer I did not meet with a single instance in which the larvæ of one colony could be mistaken for those of another, for in nearly all cases each company occupied a separate clump of nettles, and in the few cases in which more than one occurred upon a very large nettle-bed the larvæ of the different companies were at different stages of growth, and, furthermore, on different parts of the bed. In the following series of experiments the numbers III–XIII. (both inclusive) correspond to eleven companies, of which the respective larvæ were always most carefully separated.

I.—Early in the summer I began to experiment with this species. The larvæ, when found suspended in the breeding cages, were removed, and fixed against black, white, and green paper, in a strong light. The larvæ in all cases pupated shortly after transference, and there were no apparent effects wrought upon the resulting pupæ. Later investigations showed that these negative results were due to the shortness of the time during which the larvæ had been exposed to the influence of colour. The experiments also proved that the moist surface of the freshly exposed pupa is not sensitive to colour influences. A little later, I again experimented with an orange background, about twelve larvæ being kept for a large part of their lives in a cylinder lined with this colour. The pupæ were all of that very common variety which will be represented below as degree (3), and such negative results seem to prove that the species is not susceptible to such surroundings.

II.—I had asked Mr. PODÉ to procure some more larvæ of *V. Io* for me, but, as it was too late for the species, he sent a large number of *V. urticæ*. These consisted of individuals of several companies and of different ages, but in this case it was not considered safe to attempt to sort them. In estimating the pupal colours, it was

necessary to make a standard of comparison by the selection of well-marked degrees of colour, for in this species there is no distinct dimorphism, but all variations are connected by intervening forms. At this period of the investigation I arranged the pupæ according to the following standard, which was subsequently rendered more elaborate as the experiments became more precise. (Six of the most important varieties are figured in Plate 26, figs. 1-6, $\times 2$.)

- (1) Very unusually dark, almost black ; very little gold, or none.
- (2) Dark normal form ; dark grey, often with a slight pinkish tinge, with very little gold, or none.
- (3) Light normal form ; light grey, often with a pronounced pinkish tinge ; more gold than (2), occasionally none.
- (4) Lighter than (3) ; the pinkish tinge often very pronounced, and usually a large amount of gold.
- (5) Very light forms ; generally completely covered with gold.

It will be noticed that the dark colour due to pigment is shown by the above list to be developed in inverse ratio to the amount of gilded appearance, which depends upon a totally different optical condition, and pigment is absent from the gilt spots of the darkest varieties. The pink tinge forms the ground-colour of the pigment tints, while the darker forms are due to the increase in number of dark cloudy spots and the widening and multiplication of the strands of similar dark reticulations, which, in the most extreme forms, completely obscure the ground-colour. The pigment of both ground-colour and the dark spots is cuticular in position. Two degrees of pigmentation are shown in figs. 8-9, $\times 7$, Plate 26, the pupal wings alone being represented, but affording a fair criterion of the general development of pigment on the pupal surface.

In comparing the results of experiments by means of a standard, it is obvious that the classification of any series of pupæ is, at any rate, correct as affording a test of the relative amount of pigment, gilding, &c., upon the pupæ compared together at any one time. But the experiments were continued for a much longer time than that passed in the pupal stage by this species, and hence it is possible that the classification of the pupæ in the later experiments will not bear too close a comparison with that of the earlier ones. Nevertheless, I do not think that there was much difference, and when the times for taking results happened to come sufficiently near together I always classified the pupæ together, although taking notes of each company separately. But, however great be the want of parallelism between the arrangement of the initial and terminal experiments, the results would not be invalidated, for the numbers made use of in several of the series of experiments which became ready for classification together were amply sufficient to ensure conclusive results, and for each series classified together the arrangement would certainly hold good.

Mr. POND's larvae were divided into several lots, which are described as follows,

together with the resulting pupal colours, which are given in numbers corresponding to the standard list described above. The comparison was made August 19, 1886.

A. A number of larvæ were placed in a transparent glass cylinder on a white plate, which, however, soon became dark from the larval fæces. The food-plant passed through a hole in the plate into water beneath, and the top of the cylinder was covered with white muslin, much darkened and discoloured by age. Upon this disc of muslin, only 9 centims. in diameter, 18 pupæ were crowded, not one occurring in any other part. These were coloured as follows :—

| | | |
|----|------------------------------------|---|
| 4 | pupæ were (1). | . |
| 6 | „ (2). | |
| 6 | „ (3), 2 of these approaching (2). | |
| 2 | „ dead and discoloured. | |
| — | | |
| 18 | | |

It will be seen by a comparison with other experiments that the especial darkness of this lot of pupæ was due to the mutual influence of the dark bodies of the larvæ themselves hanging close together upon a limited space.

B. Another lot of larvæ were placed in a larger cylinder, but with arrangements similar in other respects. Eight pupæ were suspended from the muslin top, 1·10 decimetres in diameter, while five others were suspended from the food-plant. They were coloured as follows :—

| | | |
|---------------------------------|----|--|
| Of the 5 pupæ on the food-plant | 1 | was (2). |
| „ „ „ | 2 | were (3). |
| „ „ „ | 2 | were dead and discoloured. |
| Of the 8 „ muslin | 1 | had produced an imago, but the pupa was probably (3) ; certainly this or (2). |
| „ „ „ | 1 | was (2). |
| „ „ „ | 1 | was (4), with the gold of (5). |
| „ „ „ | 5 | were dead and discoloured. |
| | — | |
| | 13 | |

This set compares in an interesting way with the last ; being far less crowded on the larger area of muslin, there was a much smaller proportion of dark environment to each larva, and the whitish muslin could also produce its effect. Hence a much lighter series of pupæ are obtained, with more gold upon them. Comparisons of this kind led me to continue the investigations upon this species with much greater minuteness, as will be seen below.

C. Another lot of larvæ were placed in a cylinder surrounded by a single layer of green tissue-paper, which had become very yellowish-green from the action of light.

Of the 14 pupæ, 13 were suspended from the green roof—7·0 centimetres in diameter—and one from the food-plant, but the latter and seven of the former were dead and discoloured. On August 9, at 10.45 P.M., 11 individuals had already pupated on the roof, and two larvæ were suspended before pupation. At this time the roof was cut off and pinned in close proximity to a north window, so that the pupæ, &c., still hung vertically. At 10.5 A.M., August 10, these two larvæ had pupated, but evidently quite recently, for the surface of both was still greenish. Compared with the rest on August 19, these results were obtained :—

Of the 4 living pupæ which threw off the larval
skin in the cylinder 4 were (3), 2 of them with rather unusual gold, and
tending in lightness also towards (4).
Of the 2 living pupæ which threw off the larval
skin in the strong north light 1 was (3).
1 „ (4).
—
(6)

It might be supposed that the greater amount of light perhaps influenced the colour of the two larvæ which pupated latest, and which were exposed in the window for 10–11 hours, but the evidence is insufficient, as the difference between the two sets of pupæ is so small.

D. Another lot of larvæ were placed in a cylinder similar to the last. On August 9, at 10.30 P.M., the yellowish-green paper roof, 7·5 centimetres in diameter, was cut off and pinned in a north window as before. At that time four pupæ were hanging from the paper, to which nine larvæ were also suspended. By 10.5 A.M., August 10, four had pupated some hours, and two more quite recently, while the three remaining larvæ pupated at some time (unknown) later than 10.5 A.M. All these were marked and compared on August 19, the following results being obtained :—

Of the 4 which pupated in the cylinder 2 were (2).
2 „ (3).
„ 4 „ „ after a short time in the north light 1 was (1).
3 were (3), 2 of them approaching (2) very
closely and really intermediate.
„ 2 „ „ „ longer „ „ 1 was (2).
1 „ (3), with rather more gold than usual.
„ 3 „ „ „ still longer „ „ 3 were (3), 2 of them tending towards (2).
—
13

In this case it is seen that practically no effect was produced by the light, a fact which I afterwards learnt was to be expected, for a strong light merely tends towards the special coloration which follows from the illumination of the surroundings of the pupa, if such surroundings are coloured by any of the tints for which the organism is sensitive. In all the experiments with green cylinders I had in mind the green form

of *V. Io* and the green form of so many other dimorphic species (*Papilio machaon*, *Anthocaris cardamines*, &c.). But I was soon convinced that *Vanessa urticae* has no green form, and therefore in these experiments, in which green was the predominant colour, the results produced showed no fixed relation to the chief part of the surroundings, but must have been determined by individual tendencies irrespective of external stimuli, except such as were provided by the amount of shade in such covered cylinders, and by the presence of neighbouring larvæ and pupæ.

E. Another lot of larvæ were placed in a cylinder similar to the last, except that a new roof had been recently added, consisting of two layers of green tissue-paper, while the sides were surrounded by a single layer which had become very transparent and yellowish. On August 19 there were five pupæ, of which four were on the comparatively opaque and dark roof, while one was suspended from the bare stem of the food-plant and fully exposed to the yellow light coming in through the sides. The colours were :—

| | |
|---------------------------------|--|
| Of the 4 pupæ on the roof . . . | 1 was (2). |
| | 3 were (3), 1 of them with unusual gold. |
| On the food-plant 1 was | (5), splendidly golden all over. |
| — | |
| 5 | |

This experiment, more than any other in Series II., convinced me that the pupæ vary in lightness and darkness, in brilliancy and dulness, and that it was useless to continue the employment of green cylinders, but that such colours as black and white would be far more likely to yield satisfactory results.

Three larvæ were removed from this cylinder on August 13, and were treated in a manner which will be described below.

F. Another lot of larvæ were placed in a cylinder also covered with the same green paper, but in this instance there were two layers over the cylinder as well as the roof; but the paper was faded, becoming yellowish and comparatively transparent. The larvæ in this cylinder were blinded by painting over the region of the ocelli with black varnish (a quickly-drying photographic varnish, rendered opaque by the addition of lamp-black). Five pupæ were suspended from the roof, and one was lying on the floor. The colours were :—

| | |
|---------------------------------|--|
| Of the 5 pupæ on the roof . . . | 3 were (2), 1 with rather more gold than the others. |
| | 1 was (3). |
| | 1 „ (5). |
| On the floor 1 „ | (2). |
| — | |
| 6 | |

It is seen that the blinding made no difference to the result, at any rate in the direction of producing darker pupæ. This conclusion will be confirmed later.

G. Another lot of larvæ were placed in a small cylinder surrounded by two layers of black tissue-paper, and with a roof of two layers. Only a single larva reached the pupal state, but this was an exceedingly dark and well-marked (1).

This result also contributed greatly towards the frequent use of black surroundings in subsequent experiments.

H. Another lot were placed in a cylinder covered, as before, with one layer of green paper, yellowish on the sides, but recently renewed and greener on the roof. Of the seven pupæ, four were on the roof and three on the food-plant. The results were :—

| | | |
|---------------------------|-------|--|
| Of the 4 pupæ on the roof | . . | 3 were (3). |
| | | 1 was (5), with the usual extreme development of gold. |
| „ 3 „ „ food-plant | . 1 „ | (1). |
| | | 2 were (3), 1 with rather more gold than usual. |
| | | — |
| | | 7 |

There is nothing to add to what has been said before concerning the colour of pupæ in the green cylinders. On August 13 three larvæ were removed from this cylinder and treated in a manner which will be described.

I. Although all the above-mentioned pupæ in Series II. were compared together on August 19 (because by that date nearly all had reached the pupal stage), I had been watching the results seen in the various cylinders for many days, and had started further experiments, which were suggested by those already described, so that both series of results could be compared together on the above-mentioned date. The effect in the black cylinder (G.), and that in E., having convinced me that black and white would be good colours with which to experiment, it seemed clear that, if successful, there would be a more decided colour-relation between the dark pupæ and the black surroundings than between the brilliantly metallic pupæ and their white environment; and if the former relation was, as it seemed to be, most real and protective, it appeared advisable to offer the pupa a surface which would harmonise with its gilded form as completely as the black surface did with the darker variety. Having already seen a few instances of the gilding developed upon No. (5), it was quite clear that nothing could harmonise so well with it as the brilliant colour of metallic gold-leaf. On entering upon such an experiment, I was not prepared for the extraordinary success with which it was attended. But the conclusions from the other experiments led up to this as a crucial test, and it certainly seemed well worth the trial. I procured some paper covered with gold-leaf, and with it lined the inside of a box which was provided with a glass front, and was placed facing a strong east light. The side of the box, which formed the floor in this position, was covered with brown paper. A vertical partition, also gilded, separated the box into two equal compartments, which possessed every

condition in the way of illumination, &c., in common. Care was also taken that the amount and distribution of food-plant should be as nearly equal as possible in the two compartments.

(α) Thirteen larvæ were placed in one compartment, and, as eight of these were dead on August 13, three were added from the set described as H., and three from that labelled E. On August 19 there were eight pupæ, of which four were dead and discoloured, and of the others one was on the roof and three on the food-plant. The results were :—

| | | |
|----------------------------|------------|---|
| Of the 3 on the food-plant | 1 was (3). | |
| 1 " | (4) | } With the splendid golden appearance which is usual in these degrees of colour. |
| 1 " | (5) | |
| On the roof 1 | " (5) | |
| | <hr/> 4 | |

This was a very complete result as compared with those previously recorded, and left no doubt that the metallic appearance can be influenced in the most remarkable way by the use of surrounding surfaces with a corresponding colour. Such a conclusion will receive the most complete confirmation below.

(β) In the other compartment 12 larvæ were placed after having been blinded as carefully as possible. The two pupæ which were ultimately formed possessed the following degrees of colour :—

| | |
|-------------------------|-------------------------|
| 1 on the food-plant | . . . was (5). |
| 1 " floor (brown paper) | " (3), but a light one. |

Here again the blinding produced no effect on the colour as far as the evidence goes.

J. Two exactly similar white gas globes of "opal" glass were selected, and the open upper end of each was covered with white paper, each globe being placed on a perforated white plate. The lower end of the stem of the food-plant (in small and, as far as possible, equal amounts) passed through the perforation into water, while the leaves and stem above the plate were in each case introduced into the globe through its lower opening as the globe itself was lowered on to the plate. The globes were placed in a strong north light.

(α) In one globe 10 larvæ were placed, and by August 19 four had pupated upon the leaves of the food-plant in all cases, while three larvæ were still feeding (three having died).

| | |
|-----------------------|----------------------|
| Of the 4 pupæ | 1 was (3). |
| | 3 were (5), typical. |

Hence the bright white surroundings produced very striking results.

(β) 10 blinded larvæ were placed in the other globe, and five had pupated by

August 19. Of these, two were dead and discoloured, one was on the roof, and two on the food-plant.

Of the 2 on the food-plant . . . 1 was (2).

1 „ (3).

The 1 on the roof „ (5) typical.

These pupæ are certainly darker, as a whole, than the unblinded ones, but the difference is not great enough to suggest the blinding as a necessary cause, while other experiments (to follow) clearly show that it cannot have been effective in this way.

This concludes the experiments conducted upon the larvæ of Series II., kindly sent me by Mr. PODE. The great mortality was due to the fact that the larvæ were sent very young, and were thus brought up for nearly the whole period of larval life in unnatural conditions. In the following experiments I made use of larvæ which were found in various localities near Oxford, and which were allowed to remain upon the nettle-bed until they were nearly mature. In this way I secured a very high percentage of pupæ. Nevertheless, in the experiments just recorded (II.), that insight into the colour sensitiveness of the species was gained which rendered all the following experiments possible and suggested the various modifications and details.

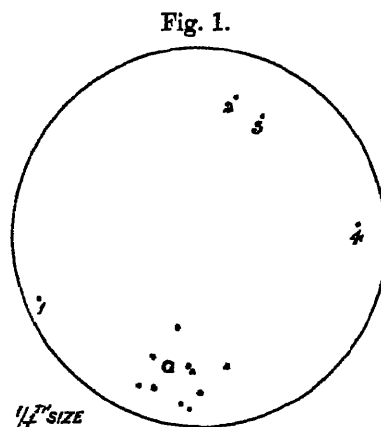
III.—A company of mature larvæ was found August 15 beside the towing-path of the “Upper River,” near Oxford. In the press of other work they were in some cases temporarily placed in short, wide, glass cylinders, covered with sheets of glass, until the night of August 16, when they were counted and to some extent re-arranged, but in the meantime they had ceased to feed; in fact, the great majority did not eat anything at all after capture. They were arranged as follows, and were all compared August 21, both together and with those of Series II.

A. A clear glass cylinder was employed, 2·07 decimetres in internal diameter and 7·0 centimetres in height. This was placed upon a floor of white paper, upon which a small quantity of the food-plant was lying horizontally, and the cylinder was covered with a sheet of white translucent “opal” glass, forming the roof. The larvæ described in this division, although first counted and noted on the night of August 16, had previously been in the same cylinder with the same white roof (although without the paper floor, as the cylinder merely rested on the ordinary floor boarding of the room). On the night of the 16th they were placed on a table about six feet from a very large window, so as to be exposed to a good east light during the day. Before this time they had been upon the floor of the room under the shadow of the table. When re-arranged on the 16th, 11 larvæ were hanging from the roof preparatory to pupation, and by 4 P.M. on the 17th four had pupated and were removed, and by the same evening seven more had also changed. When compared on August 21st, there were 15 pupæ arranged and coloured as follows:—

- 10 suspended from the roof in a smallish group { 4 removed Aug. 17. of which all were light (3), 3 with more gold than usual but not the gold of (4).
6 remained hanging, of which 5 were . . (3), 2 lightish.
1 was . . (4).
- 4 isolated, suspended from various parts of the roof, of which 2 were . (4), 1 with unusual gold, but not equal to (5).
2 „ . . (5).
- 1 isolated, lying on the white floor, not attached to food-plant; it was . (4).

15

The relative positions of the pupæ are shown in fig. 1 ($\frac{1}{4}$ -size).



The circle represents one-fourth the size of the white-paper roof, looked at from below, so that the points of attachment of the pupæ are seen (indicated by the 14 black dots). The dots 1-4 mark the positions of the 4 isolated pupæ. The 10 dots at G similarly show the positions of the 10 pupæ, which were arranged in a compact group. Each dot corresponds to the position of the boss of silk to which the pupæ was fixed.

Various results come out very clearly from this experiment :—

(1.) The larvæ were captured on the afternoon of August 15, and 11 out of 15 had pupated by the evening of August 17, while seven of these pupated between the afternoon and evening of this day. I do not think that any of these larvæ ate anything after capture; it is quite safe to assume that the 11 which first changed did not feed. It is therefore probable that by far the greatest part of the period intervening between capture and pupation (about 48 hours) corresponded to the normal period which intervenes between the cessation of feeding and pupation. I have frequently noticed that when mature larvæ, almost ready to cease feeding, are captured in the field they do not eat at all in captivity, but immediately prepare for pupation, the change appearing to be slightly hurried on by the shock given to the larva. In this species the period between the cessation of feeding and pupation, which will in future be called the "preparatory period," consists of three stages :—

Stage I., in which the larva quits its food-plant and hurries about, seeking for some place upon which to pupate.

Stage II., in which the larva rests motionless upon the selected surface and towards the end of the stage spins the boss of silk for its subsequent suspension.

Stage III., in which the larva hangs suspended by its posterior claspers from a boss of silk.

It is to be noted that Stage I. must be of very indefinite length, depending chiefly upon the varying proximity of places suitable for pupation. It will be shown that if such suitable surfaces are not reached the larva finally makes the best of anything which happens to be near, or often pupates in a horizontal position without suspending itself. Under ordinary circumstances the larva is exposed to the effective colour influence during Stages II. and III. only, for in the previous stage it is wandering over surfaces of various colours; hence the pupal colour must, as a rule, be determined in the two later stages. Further experiments will show that these important stages are of more constant length than the first stage.

(2.) The experiment shows the great power of the white surface in producing light and gilded varieties.

(3.) It shows the great influence of closely adjacent, but comparatively small, dark objects in modifying the effect which would have followed from a white surface. Thus, of the 10 larvæ, arranged in a small group so that the dark-skinned component individuals were exposed to mutual influence, 9 were (3) and 1 was (4), while of the 5 larvæ isolated upon the roof and floor 3 were (4) and 2 were (5). No importance is to be attributed to the removal of the four first-formed pupæ on August 17 (afternoon), because any effect produced by them on their neighbours must have been wrought before the transference took place.

B. Another set of larvæ were blinded, and (August 16, evening) placed in a cylinder of almost the same size (2.16 decimetres in internal diameter, and 1.02 decimetres in height), with a similar roof and floor and amount of food, and the same conditions of light. It is, however, to be noted as very important that these larvæ had not been previously exposed to the influence of a white roof, but were taken from a cylinder covered with a sheet of green glass, the whole being placed upon the floor in the shadow of the table. To compensate for this, the least mature larvæ were selected, *i.e.*, those which, as far as possible, were feeding, or had not passed beyond Stage I. of the period before pupation. Nevertheless, the comparison with A. is unsatisfactory, because in selecting the least advanced larvæ out of very large numbers the results are liable to be influenced by the fact that such larvæ are often less healthy than the others, and frequently do not ultimately attain the average size. On August 21 the following results were obtained :—

| | |
|---|---|
| 3 lying on the floor, not attached to the food | $\left\{ \begin{array}{l} 2 \text{ deformed and with larval heads attached, both} \\ \text{apparently} \dots \dots \dots \text{light (2).} \\ 1 \text{ normal} \dots \dots \dots \text{light (2).} \end{array} \right.$ |
| 3 suspended to the glass side of cylinder | $\left\{ \begin{array}{l} 2 \text{ deformed as above, both apparently.} \dots \dots (5). \\ 1 \text{ normal} \dots \dots \dots \text{light (3).} \end{array} \right.$ |
| 2 suspended to the roof (about 1.9 centimetres apart) | both (4), but with little gold for this stage. |
| <hr/> 8 | |

The deformity is due to the fact that the larvæ, when blinded, dash their heads about, and so tend to spread the varnish over a much larger area, and it is thus liable to harden on the top of the head, and to prevent the latter from splitting in the complete manner which is necessary for ecdysis.

I have been obliged to quote an "apparent" degree of colour for the deformed pupæ, because it was difficult to judge them by the same standard as the normal ones. In the result it is to be noted that the white roof produced a strong effect on the pupæ suspended from it, and that those attached to the side of the low cylinder also came under its influence. The floor was white, but the relative proximity of the dark leaves and stems of the food-plant may have made all the difference. On the other hand, these pupæ were not crowded together, as in the majority of the A. division. This last important difference in arrangement, which must certainly produce some considerable effect upon colour, together with the possible sources of error introduced by the food necessary for many larvæ, induced me to alter the conditions of these comparison experiments in a manner which will be described below.

Allowing for all uncertainties, there is no sufficient ground in the respective results of these two divisions (A. and B.) for the belief that the ocelli represent the larval sensory surface through which the colour influence makes itself felt.

C. Nearly all the remaining larvæ were placed (August 16, evening) in a large cylinder (2.39 decimetres in internal diameter and 1.02 decimetres in height) covered with one layer of black tissue-paper, and with a similar roof and floor. Thus, although the surrounding surfaces were all black, a considerable amount of light could enter the cylinder. These larvæ had previously been in the green-glass covered cylinder from which those of the B. division were taken. Compared with the others, and with Series II., the results were:—

| | |
|---------------------------|--|
| 3 suspended from the roof | $\left\{ \begin{array}{l} 2 \text{ near together, both (3), one of them light (3).} \\ 1 \text{ isolated and} \dots \dots (3), \text{ darkish.} \end{array} \right.$ |
| 1 suspended from the side | ... (4). |
| 2 on the floor | ... were both (2). |

6

Further experiments will show that these results were largely due to the exposure previous to the evening of the 16th. I was unable to take notes of the hours at which suspension or pupation took place in this or the B. division, but, as the latter

consisted of the least advanced larvæ, very carefully selected, it follows that the remainder, which (with two exceptions, forming D division) formed this division, were comparatively mature and doubtless far advanced in Stage II. of the preparatory period when the black roof was added. Nevertheless the results are important when taken with others, as showing that the influence works upon the larva for some considerable time before pupation, and therefore, when it is only introduced shortly before the change, great effects are not produced; but the same conclusion will be reached by far more careful experiments, to be described below.

D. Two larvæ were already suspended from the green glass cover alluded to above on the evening of the 16th. The glass was placed in a vertical position in a strong north light, the larvæ being on the side towards the room. On comparing, their colours were :—

2 pupæ, both (3), 1 darkish and 1 lightish.

Thus 31 pupæ were obtained from Series III., and I do not think that any of the larvæ died. I did not, at the time, know the number of larvæ in a company of average size, or it would have been clear that the captured larvæ were merely the remnant of a company of which the large majority had already sought pupation. Being greatly in want of material, I searched very carefully, and, I believe, obtained all the larvæ left on the nettle-bed. Had I known that the larvæ were in this position, and therefore almost certain to pupate directly, I should have acted differently. Further experiments will show that, to obtain sufficiently accurate results, the most exacting demands are made upon time and at a moment's notice; for as soon as the material is obtained the first few hours may prove to be the most important of all.

Nevertheless, the conclusions from A. division are most interesting, and thoroughly borne out by other work; and, allowing for all sources of error, the comparison between A. and B. is also satisfactory.

IV.—Another company of mature larvæ was found, also on August 15, on a large patch of nettles in Binsey churchyard. On the evening of the 15th these were placed in two cylinders, one very large and covered with a sheet of clear glass, the other smaller and covered with a sheet of "opal" glass. Both were on the floor, in the shadow of the table. On the next day they were re-arranged according to the following divisions. Comparison of the pupæ, together with those of all previous series except I., took place August 22.

A. In the afternoon of the 16th 12 larvæ were taken from the large cylinder, and were placed in a small cylinder (6·2 centimetres in internal diameter; 1·18 decimetres in height) covered with two layers of black tissue-paper, with a similar roof, also of two layers, and a black floor. The larvæ which were selected had not yet entered

Stages II. or III. of the preparatory period. All the 12 pupated upon the roof, and were therefore crowded close together on a black disc 6·2 centimetres in diameter. The results were:—

| | | |
|------------------|--|---|
| Of the 12 pupæ . | 3 were (1), not very decidedly black for this division | } Hence these were very hard to separate. |
| 4 „ | (2), decidedly dark for this division | |
| 5 „ | (3), 4 of them dark (3), one normal, but in all 5 there was the normal gold and pink ground-tint; but the dark pigment, where present, was much blacker than in the normal (3), so that these 5 presented a very different appearance from the (3) of other cylinders. | |

It was interesting to note that seven out of the 12 were much darker than the darkest of Series III. By re-arranging these larvæ a few hours earlier on the 16th, and by selecting the least advanced out of a larger number, the black surroundings seem to have had the opportunity of producing their full effect; but it is also probable that these larvæ were more easily influenced in this direction.

B. The large cylinder (3·66 decimetres in internal diameter, and 1·34 decimetres in height), covered with a sheet of clear glass, already mentioned, was left in the same place on the floor, and in the evening of the 16th, when many larvæ were advanced in Stage II. upon the roof, a sheet of black paper was placed over part of the latter, on its less shaded side, while a sheet of white paper was placed over the more shaded part. As the roof was formed of clear transparent glass, there was every reason for believing that the same effect would be produced as if the roof had been really formed of black and white paper. All the larvæ pupated on the glass roof in three groups, and their colours were as follows:—

Group 1 was under the black paper and consisted of 25 pupæ,

| | |
|--------------------|--------------------------------------|
| of which | 5 were (1). |
| | 14 „ (2). |
| | 5 „ (3), dark. |
| | 1 was (3), with the gold of (4), but |
| | the pigment very dark. |
| | <hr/> 25 |

Comparing these with the pupæ of A. division, the (1) were alike, the (2) of A. were a little darker; the dark (3) of A. were rather lighter, and the curious (3) differed in that from A. being more pink, that from B. more gold. There was very little difference between the dark (2) of A. and the (1) of B., which are not at all extreme, although the resemblance is chiefly due to the especial darkness of the (2). The amount of very dark pigment on the (3) of both A. and B. brings them very near the (2), but the prominent pink ground-tint is inconsistent with the latter degree.

Group 2 was under the black paper and consisted of 11 pupæ, of which 3 were (2).

| | |
|-------|----------------|
| 8 „ | (3), all dark. |
| <hr/> | |
| 11 | |

A very uniform group. The (2) and dark (3) resemble those described above, and have the same relation to the A. division.

Group 3 was under the white paper, and consisted of 5 pupæ, of which all were (3), dark.

All exactly resembled those described above under the same degree.

No less than 22 out of the 41 pupæ were darker than any of Series III., and, omitting the four (2) of the latter, all the pupæ in Divisions A. and B., except the two lightest (3), are darker than any in the preceding series. There were pupæ among the dark (3) in Series III. with a greater amount of pigment than some of the dark (3) in the A. division, but the pigment in the latter was much darker in colour, although their pink ground-tint was also more distinct. It is, however, perhaps safer to exclude the five (3) of A. (IV.) from the above general statement as to the comparative darkness of these divisions and of Series III.

The comparison instituted above seems to show that these larvæ tend, as a whole, to produce darker pupæ than those of Series III. Such a conclusion shows how important it is in these experiments to keep the larvæ of the different companies carefully separate, for the error due to the different tendencies largely disappears in the experiments conducted within the limits of a single company. It appears that the white paper added when the preparatory period was far advanced nevertheless produced some slight effect, for none of the pupæ suspended under it were darker than dark (3), although on the more shady side of the cylinder.

C. Some of the larvæ from the other smaller cylinder, covered with "opal" glass, were (August 16, evening) placed in a cylinder (2.64 decimetres in internal diameter, and 9 centimetres in height) which had a roof of white glazed paper and a floor of ordinary white paper (common unruled white foolscap was always used for the white paper floors). This was placed in a good east light, about 5 feet from a large window. None of the larvæ in the former cylinder were suspended when the change was made, but many were prepared for suspension. The following results were obtained:—

| | |
|--|---|
| Of 3 pupæ on the floor, but not fixed to the | |
| food-plant | 2 were (3). |
| | 1 was (4). |
| „ 6 pupæ in a small group suspended from | |
| the roof | all were (3), 1 of them dark (3). |
| „ 6 pupæ in another small group, suspended | |
| from the opposite side of the roof . . . | 1 was (2). |
| | 5 were (3), 4 of them light, 1 with unusual gold, 8 |
| | with unusual pink. |
| „ 1 pupa, suspended to the side close to the | |
| — last group, | was (3), dark. |

16

The results show clearly that the larvæ of this series were greatly influenced by white surroundings, for the above list is a very sharp contrast with either of the

divisions A. or B. At the same time, the influence was not nearly so strong as that of the same colour applied at the same time to Series III. (A. and B.). So that there is further support for the conclusion that the latter series tended more strongly towards the light forms than the larvæ of the company now being described.

D. Another lot of larvæ were taken (August 16, evening) from the smaller cylinder, those being especially selected which were as far removed as possible from suspension. They were placed in a cylinder (1·85 decimetres in internal diameter, and 8 centimetres in height) covered with a roof of gilt paper, which descended down the sides of the cylinder for a distance of about 1·6 centimetres. The floor was of white paper, and the conditions of light were similar to those of division C. The following results were obtained :—

| | |
|---|--|
| Of 3 pupæ on the floor, but not fixed to the food-plant | 2 were (3), 1 of them dark and 1 light. 1 was (4), very golden. |
| „ 2 pupæ on the glass side, about halfway between the edge of the gilt paper and the floor, and close together (about 1·3 centimetres apart). | both were (3), 1 of them light, with unusual gold for (3). |
| 1 pupa on the gilt roof, just above the two last, | was (3). |
| 1 „ isolated on the roof | was (5), with plenty of gold, but not so extremely bright as is common. |
| Of 4 pupæ in a little group on the roof . . . | 3 were (3), 1 more pinkish than usual, 1 rather more golden; but neither could be called light (3). 1 was (2), very dark; almost a (1). |

11

These results are very similar to the last, and suggest the same conclusions. In both there is some evidence for the effect of the larval bodies upon one another during the preparatory period.

E. (α) On the same evening (August 16) six larvæ, as far removed as possible from suspension, were chosen from the smaller cylinder and placed in an “opal” globe similar to that previously described (II., J.), with a glazed white paper roof and white paper floor. This was placed in a strong north light. When compared on the 22nd, the pupæ had the following arrangement and colour :—All the pupæ were suspended high above the food, and near the top of the globe upon its overhanging sides, although none of them were fixed to the roof.

| | | |
|--|------------------------------------|--|
| The most isolated pupa was . 5.4 centimetres from the one nearest to it | | and it was (4), very golden. |
| The pupa next in degree of isolation was | 5.4 centimetres from the last, and | It was light (3), unusually pink. |
| | 8.4 " " another pupa | |
| The pupa next in degree of isolation was | 3.4 centimetres from the last, and | It was light (3), unusually golden. |
| | 4.0 " " the nearest of the group. | |
| The 3 remaining pupæ were arranged in a group, at the angles of an isosceles triangle of which the sides were about 1.3 centimetre in length and the base about 2.1 centimetres. | | They were all (3), 2 of them dark, although with pink colour distinct. |

The effects produced by the white surroundings are, perhaps, rather stronger in this case, and are to be accounted for by the especial care with which the less mature larvæ were selected, so that the influence was of longer duration. The delicate susceptibility of the larvæ to the dark bodies of others near to them is extremely well shown in the difference between the three isolated pupæ and the three in a small group, and also in the difference between the most isolated pupa in the former number and the other two.

(β) At the same time six exactly similar larvæ were selected from the same cylinder, blinded, and placed in an "opal" globe, under precisely similar circumstances of food, light, &c. The results were:—

| | | |
|--|--|---|
| Of 3 pupæ lying on the floor, but not attached to the food, | | 2 were deformed, and were apparently 1 dark (3), 1 light (3). |
| | | 1 was perfect, and was (3). |
| ,, 2 suspended more than halfway up the sides of the globe and 1.05 decimetre apart, both were | | (3). |

In the relative results of (α) and (β) there is no sufficient reason for believing that the blinding produced any effect. Three of the blinded larvæ pupated in close proximity to the dark food, and therefore were in this respect under darker influences than the unblinded larvæ; but, on the other hand, the latter were more crowded, and so were under darker influences from another cause. Such an uncertainty, introduced into an experiment conducted with considerable care, chiefly induced me to separate the larvæ to a far greater extent, so as to diminish the errors due to both causes.

Thus 91 pupæ were obtained from Series IV., and very few of the larvæ died. It is, therefore, likely that I had obtained nearly the whole of a smallish company, and that none of them had previously left the nettle-bed. The results are open to the criticism upon Series III., but to a rather less extent, because, the larvæ being slightly less advanced, there was more opportunity for the influence of the coloured surroundings when the re-arrangement took place on the evening of August 16. The

demonstration of a somewhat different tendency in the larvæ of this company, as a whole, from those in the preceding company is of importance.

V.—The remnant of a company was found (August 22) on a very large nettle-bed close to South Hincksey. From the extent over which the larvæ ranged, and the amount of nettle which had been eaten, it was evident that the company had been very large. Professor BURDON SANDERSON was with me at the time, and I spoke of the negative results of the blinding experiments, and asked his opinion as to the possible presence of the desired terminal organ in connection with the large and complex bristles which are present on the larvæ. As a result of our conversation, I determined to devote the greater part of these larvæ to the investigation of this question. I also wished, by the careful study of a few larvæ of this company, to ascertain as accurately as possible the duration of the period between the cessation of feeding and pupation, and of its constituent stages, especially II. and III. It was to be expected that Stage I. would be abnormally short, as the larvæ were confined in so limited a space.

The stock of larvæ were placed at 10.30 P.M., August 22, in the two cylinders mentioned in III., A., and III., B.; similarly arranged, with "opal" roofs and white-paper floors, but placed so as to receive a strong east light just beneath a large window. The experiments are described below under their respective divisions.

A. On the evening of August 22 a few larvæ were selected to form the subjects of experiment to test the above-mentioned suggestion as to whether the large branching spines contained any terminal organ which received impressions from coloured surfaces, and was thus the means of modifying the pupal colours. There are seven of these spines on most of the segments, and when they were snipped off the bases bled a little, and so it was clear that a subcuticular core was contained within them.

(α) Four larvæ, apparently still feeding, were selected, and their bristles were carefully snipped off, being cut as near to the base as was consistent with safety. These larvæ were placed in one of the two compartments of the gilt box already described, which faced a strong east light, standing about two feet from a large window. The history of the larvæ is given below:—

| | | |
|---------------------------|--|---|
| Aug. 22. 10 30 P.M. . . . | 4 shorn larvæ placed in compartment. | Stage I. of preparatory periods. Stages I and II. of preparatory periods. Stages II. and III. of preparatory periods. |
| " 23. Morning . . . | Very little food eaten, and larvæ crawling up sides and on roof. | |
| " " Early afternoon | Larvæ are crawling up sides and sitting on roof. | |
| " " 6 P.M. . . . | 2 suspended, and apparently another nearly ready. | |
| " " 9.30 P.M. . . . | 2 more suspended. | |
| " 24. 2.43 P.M. . . . | 3 have pupated some little time: an hour or two. If the 3 larvæ ceased feeding about the time when they were placed in the compartment, it would make the whole preparatory period about 38-39 hours. | |
| " " 2.47-2.53 P.M. . . | The last was seen to pupate at this time. The two larvæ which were last to enter Stage III. may be assumed to have become suspended about 7.45 P.M., Aug. 23, and hence Stage III. lasted 19 hours in one case and 17-18 hours in the other. | |

Of the pupæ, two were in a corner of the roof, only 5 mm. apart, the third being 2.7 centimetres away from the one of the first-mentioned pupæ which was nearest to it, while the fourth was similarly 2.4 centimetres removed in another direction. Thus all four pupæ were suspended from the roof. Their colours were compared August 26, 4.15 P.M. :—

Of the 4 pupæ 1 was a very light (3) coming near a (4).
 2 were (4), very brilliantly golden.
 1 was. . . . (5) " "

4

(β) Five similar normal larvæ were at the same time placed in the other compartment of the gilt box. One was removed on the 24th (see the B. division), while still feeding; so that it is not necessary to describe it below. Their history is as follows :—

| | | |
|------------------------------|--|--|
| Aug. 22. 10.30 P.M. . . . | 5 normal larvæ placed in compartment. | Stages I. and II. of the preparatory period. |
| " 23. Morning and afternoon. | The larvæ have been upon the roof most of the day, except one, which was removed on the 24th. | |
| " " 9.30 P.M. . . . | 1 is suspended. | Stage III. of the preparatory period. |
| " 24. 2 A.M. . . . | Same. | |
| " " 8.35 A.M. . . . | 3 suspended; but 1 has come down from the roof, and is wandering about actively. | |
| " " 2.43 P.M. . . . | 1 has just pupated, and quite fresh. Assuming the larva to have ceased feeding about the time when it was placed in the box, the preparatory period would be about 40 hours, and if suspension took place about 7.45 P.M., Aug. 23 (as is most probable), Stage III. would have lasted 19 hours. | |
| " " 11.45 P.M. . . . | 2 more pupated, 1 recently, the other in the last few hours. If suspension began about 5.15 A.M., Aug. 24, the length of Stage III. would be about 18½ hours in one case and a few hours less in the other. | |
| " 25. 8.40 A.M. . . . | The last has pupated some hours. | |

All four pupæ were on the roof—three in a line along the right side, the anterior pupa 3·2 centimetres from the middle one, and the latter 2·2 centimetres from the posterior one. The fourth was on the other side of the roof, beside the vertical partition, and 9·5 centimetres away from the nearest of the others. Thus all were rather isolated, especially the last, but it was in a position which was on the whole less strongly illuminated. The colours were compared at the same time with the (α) sub-division :—

Of the 4 pupæ the 1 by the vertical partition was light . (3).

The 3 others by the side of the box were all (4), very golden, but not equal to the (4) pupæ of the shorn larvæ, except in one case, which is near a (5).

—
4

The comparison between these two sets is, as far as it goes, completely destructive of the theory that the bristles contain the terminal organ which was sought for. The pupæ of the shorn larvæ are on the whole rather the more brilliant. Both sets show well the extremely powerful influence of the gilded surface upon the larvæ. There are also certain data from which we can arrive at a fairly accurate estimate of the length of the preparatory period and of Stage III.

B. On the morning of August 23 the two cylinders containing the stock of larvæ were examined, and it was seen that one larva had left the food, and was resting on the roof. This larva was joined by five others about 11 A.M. They were all sitting motionless (Stage II.), but when removed and placed on fresh food they would sometimes eat a little. At noon on the 23rd five larvæ were removed from the two "opal"-roofed cylinders, and were placed in separate cylinders of nearly the same height, and all of the same internal diameter, i.e., 6·1 centimetres. Two of these larvæ (numbered 1 and 2) were among the six larvæ which had already entered Stage II. Each cylinder had a roof of white glazed paper, and two-thirds of the circumference was also surrounded (externally) by the same material, while the floor was of ordinary white paper. The cylinders were placed with the clear uncovered side facing a strong east light, about two feet from a large window. On the floor of each a single nettle-leaf was placed, which was renewed, if necessary, but always removed when the larva had ceased to feed. Thus the conditions appeared to be as nearly equal as it is possible to make them. The cylinders were numbered 1—6, and the larvæ were known by corresponding numbers. In comparing the results, it must be remembered that the larvæ numbered 1 and 2 were taken from the *roof* of the large cylinders in which the stock was kept, while those numbered 3, 4, and 5 were taken from the *food-plant*, Number 6 being added much later, and taken from the food-plant in one compartment of the gilt box previously described. The chief object of the experiment being to determine the lengths of the three stages of the period preparatory to pupation, the cylinders were frequently examined, the results being shown in a tabular form below :—

| | Cylinder 1. 1.01 declm. high. | Cylinder 2. 1.06 declm. high. | Cylinder 3. 8.7 centim. high. | Cylinder 4. 8.0 centim. high. | Cylinder 5. 8.7 centim. high. | Cylinder 6. 8.0 centim. high. |
|--------------------|--|--|----------------------------------|---|--|---|
| Aug. 23. 12 noon | Experiment began | Experiment began | Experiment began | Experiment began | Experiment began | |
| 1 P.M. | Nothing eaten | Nothing eaten | Still feeding | Still feeding | Still feeding | |
| 6 P.M. | Gone to top of cylinder | Gone to top of cylinder | Gone to top of cylinder | Gone to top of cylinder | " " | |
| 9.30 P.M. | No change | No change | No change | No change | Gone to top of cylinder | |
| 11 P.M. | " " | " " | " " | " " | No change | |
| Aug. 24. 1.10 A.M. | Suspended and placed in darkness 1.30 A.M. | " " | " " | " " | " " | |
| 2.50 A.M. | Not examined | Suspended, and probably earlier, but unnoticed | " " | " " | " " | |
| 3.45 A.M. | Still suspended | Still suspended | " " | 8.35 A.M., suspended and placed in darkness | 8.35 A.M., no change | 9.15 A.M., experiment began; 12 noon, gone up cylinder. |
| 2.53 P.M. | " " | " " | 2.43 P.M., suspended | 2.50 P.M., still suspended | 2.43 P.M., suspended; at 2.50 P.M., placed in darkness | 2.43 P.M., suspended. |
| 5.35 P.M. | Not examined | Just pupated, still moist | " " | Not examined | Not examined | " " |
| 11.30 P.M. | Just pupated, light green | " " | 11.35 P.M., still suspended | Still suspended | Still suspended | " " |
| Aug. 25. 8.40 A.M. | " " | " " | Recently pupated, still green | 8.50 A.M., pupated some hours, and darkened | 8.50 A.M., pupated lately | Recently pupated, still green. |
| Results . . . | Light (3) | Light (3) | (3) Very little gold | (3) Normal gold | (3) Very little gold | Very light (3), with rather more gold than usual. |

From the data the approximate duration of the respective stages was estimated by taking the mean of the times between which the beginnings and ends of the stages occurred, the following results being obtained :—

| | Stages I and II. | Stage III. | The whole preparatory period. |
|----------|--|---------------------------------------|--|
| Larva 1 | Incomplete | About 23 hrs. | Incomplete |
| " 2 | Incomplete | Uncertain | Incomplete |
| " 3 | About 20½ hrs. | About 20½ hrs. | About 40½ hrs. (very nearly correct) |
| " 4 | " 14½ " | " 22½ " | About 36¾ hrs. |
| " 5 | " 16 " | " 20½ " | About 36½ hrs. (very nearly correct) |
| " 6 | Incomplete | " 19 " | Incomplete |
| Averages | Average of the 3 instances 16½ hrs. | Average of the 5 instances 21 hrs. | Average of the 3 instances 37½ hrs. |

Comparing these averages with the estimates in Division A. of this series, the length of Stage III. is here rather longer, and that of the whole period slightly shorter, but without much difference. It is to be noted that in three cases (Nos. 1, 4, and 5) Stage III. was passed in the dark, and that the duration of the stage was rather longer than in the other larvæ (except in one case, in which the length was the same as that of the shortest of the stages passed in the dark). If the stages were somewhat protracted by this treatment, of course the whole preparatory period would be correspondingly lengthened. Evidence in favour of such protraction will be found in some of the later experiments upon this species, and also in the case of the *Pieridæ*.*

The exceptionally short duration assigned to Stages I. and II. in Larva 4 may be partially explained by supposing that the stage really began very soon after 1 P.M. on August 23 (instead of at 3.30 P.M., and thus halfway between 1 P.M. and 6 P.M.), and that it was thus about two hours longer. Such a supposition is rendered probable by a comparison of the times of pupation of Larvæ 3 and 4.

In such small cylinders the larvæ wandered very little before fixing on the position in which to suspend themselves, and therefore Stage I. was reduced to a minimum.

As to the colours of the pupæ, there was very great uniformity, Pupa 6 being the lightest, then Pupæ 1 and 2, and then, after an equal interval, Nos. 3, 4, and 5; but the whole of the difference being comprised in the slight interval between normal (3) and very light (3). Thus the white-paper back-ground produced much less effect than the gilt back-ground in Division A., and the lightest of these pupæ, 6, had already passed Stage I. and much of Stage II. in the gilt box. The removal of three of the larvæ into darkness during Stage III. produced no apparent effect, but the numbers

* This probable effect of darkness appeared to be so important that I experimented upon 44 larvæ during the past summer (1887) with the object of testing the above-mentioned conclusion. The larvæ, placed in a strong light, were surrounded in some cases by gilt and in others by tin surfaces; those in the dark being surrounded by black paper. Without giving the details of the experiment, I may say that its results conclusively proved that darkness does considerably protract the preparatory period. There did not appear, however, to be any evidence for the supposition that the gilded pupæ pass through a shorter preparatory period than those which are less brilliant, when both are equally exposed to light.—September 10, 1887.

employed, together with their results, are insufficient evidence from which to conclude that the larvæ are not sensitive during this stage, although it appears almost certain that the earlier Stage II. is the time of chief susceptibility to surrounding influences. It will be shown that the larva can hardly be susceptible after the first part of Stage III., and it is very likely that in two out of three instances the most important and earliest hours had already elapsed when the larvæ were transferred; this suggestion cannot, however, explain the case of No. 1, for this larva must have been shifted very soon after the beginning of suspension.

C. Another division of larvæ was taken from the "opal"-roofed cylinders and made use of in order to test whether the spines contain the terminal organ which receives impressions from coloured surroundings. Nine larvæ were carefully shorn of their bristles on the evening of August 23, and two of them were left in one of the above-described "opal"-covered cylinders as they had quitted the food and were resting on its roof, while the other seven, being rather less advanced, were placed one in each of seven "opal" globes similar to those previously described, except that a glazed white-paper roof was, in nearly all instances, fixed to the smaller opening, while the edge of the larger opening of the globe rested on a floor of ordinary white paper, upon which never more than two leaves were placed at one time, renewed when necessary, and removed when it was obvious that the larva had ceased to feed. Seven exactly similar, but normal, larvæ were placed in seven other globes, all conditions being identical, except the following unimportant differences, which were compensated as far as possible:—The globe numbered 1 below was lower and smaller than the others; that numbered 2 was turned with the larger end uppermost and covered by the roof; 5 and 13 had been broken and were mended with white glazed paper, so that there was more surface of paper in 5 than in the others, the loose piece in 13 being merely retained in its place by paper glued on to the outside of the globe. The 14 globes were arranged on two shelves, one above the other, in a strong north light close to a large window, and the two series of larvæ were placed in alternate globes: the shorn larvæ occupying globes with the even numbers 2–14, the others occupying those with the odd numbers 1–13; and Globes 1–7 occupied the upper shelf, 8–14 being below. By this arrangement all possible errors due to differences of illumination were compensated, for as soon as a larva had ascended the side or had suspended itself the globe was always turned round, so that the larva was next to the window. Such an experiment was certain to yield useful results, apart from the main question of a possible terminal organ in the bristles; for, by noting the results of frequent examination, it was possible to gain further knowledge of the three stages of the preparatory period; and, as in the larvæ of Division B., the action of white surroundings upon the larvæ was tested in the most satisfactory way by the elimination of the sources of error present in the otherwise similar experiments of the preceding series. The results of examination are expressed in a tabular form below. The larvæ numbered 4 and 5 died, and are not further alluded to.

| | Globe 1. Normal. | Globe 2. Shorn. | Globe 3. Normal. | Globe 6. Shorn. | Globe 7. Normal. | Globe 8. Shorn. | Globe 9. Normal. | Globe 10. Shorn. | Globe 11. Normal. | Globe 12. Shorn. | Globe 13. Normal. | Globe 14. Shorn. |
|---|--|--------------------------------------|---------------------------------|-----------------------------|--------------------------------|-----------------------------------|------------------------|------------------------------|--|---------------------------|---------------------------|------------------------------|
| Aug. 23, 10 15 P.M. | Experiment began | Experiment began | Experiment began | Experiment began | Experiment began | Experiment began | Experiment began | Experiment began | Experiment began | Experiment began | Experiment began | Experiment began |
| Aug. 24, 9 25 A.M. | Very little eaten; gone up globe | Still feeding | A little eaten, suspended | Little eaten | Nothing eaten; suspended | Still feeding | Much eaten; gone up | Still feeding | Still feeding | Little eaten; gone up | Still feeding | Still feeding |
| 3 P.M. | Suspended | Gone up globe: spun hairs | " | Gone up | " | Gone up | Came down | " | " | Suspended | " | " |
| 7.54 P.M. | " | Suspended | " | Suspended | " | " | Gone up | " | " | " | Gone up | Gone up |
| 11 35 P.M. | " | " | Just pupated; still green | " | Just pupated; still green | " | Suspended | Gone up | " | " | " | " |
| Aug. 25, 8.40 A.M. | Pupated; darkened | " | " | " | " | Suspended | " | " | " | " | " | Suspended |
| 6.55 P.M. | " | Pupated; darkened | " | Pupated, darkened | " | " | Pupated; darkened | Suspended | " | Pupated; darkened | Suspended | Just pupated; still green |
| 10 40 P.M. | " | " | " | " | " | Pupated, partially darkened | " | " | " | " | " | " |
| Aug. 26, 10 A.M. | " | " | " | " | " | " | " | Just pupated; still green | Pupated at much later date | " | Pupated, darkened | " |
| Position of pupæ | Side of globe, ½ up | Side of globe, just below roof | Side, rather over ½ up | Side, 3/8 centims. up | Side, 3/8 centims. up | Side, ½ up | Side, ½ up | Side, ½ up | Side, height unnoted | Side, rather over ½ up | Side, rather over ½ up | Side, ½ up |
| Colour of pupæ, Aug. 26, 3.30 P.M. | (4) Much bright gold | (4) Much bright gold | (1) Much bright gold | (3) Light | (3) Light | (3) Light | (3) | (3) | Very light (3) (compared Aug. 20) | Light (3) | (3) | (3) |

From this Table the duration of the stages can be approximately estimated as follows :—

| | Stages I. and II. | | Stage III. | | The whole period. | |
|----|-------------------|-------|------------|-------|-------------------|-------|
| | hrs. | mins. | hrs. | mins. | hrs. | mins. |
| 1 | About 8 | 22 | About 15 | 50 | About 24 | 12 |
| 2 | " 5 | 15 | " 20 | 20 | " 25 | 35 |
| 3 | " 5 | 0 | " 19 | 10 | " 24 | 0 |
| 6 | " 5 | 15 | " 20 | 20 | " 25 | 35 |
| 7 | " .. | .. | " 19 | 10 | " .. | .. |
| 8 | " 15 | 50 | " 17 | 0 | " 32 | 45 |
| 9 | " 4 | 18 | " 16 | 0 | " 20 | 20 |
| 10 | " 16 | 0 | " 19 | 45 | " 35 | 45 |
| 12 | " 8 | 22 | " 25 | 35 | " 34 | 0 |
| 13 | " 20 | 20 | " 14 | 30 | " 35 | 0 |
| 14 | " 10 | 35 | " 14 | 30 | " 25 | 0 |

The comparison between these pupæ and those of Division B. was most carefully carried out, and the two divisions were compared together. The method adopted in comparing the results of all careful experiments was as follows. The pupæ were arranged side by side on a sheet of white paper, so that all their ventral surfaces were illuminated by a strong east light which fell upon all the pupæ at the same angle; having thus decided upon their arrangement in the order of relative darkness or amount of gilding, they were all turned over, or the paper turned round so that the dorsal surfaces were illuminated in the same manner; and the previous order was confirmed or modified, as the case might be; but on very nearly all occasions the two surfaces gave corresponding results, and the only exceptions were when the differences were extremely slight. The results of the experiment tabulated above agree with those of Division A. in being destructive of the supposition that the desired *terminal organ exists in the larval bristles*. The figures seem to be very conclusive on this point. As to the length of the stages and of the preparatory period brought out by the above Table, there is little doubt that the larvæ had in nearly all cases begun the period before being placed in the globes, and that the estimated length of the whole period and that of the two initial stages is far too short in nearly all cases. Further evidence of this suggestion will be adduced in the next division (D.). It is probable that the estimated length of the last stage (III.) is, on the whole, about normal, as the above 11 instances give an average length of 18 hours 16 $\frac{4}{11}$ min. for this stage. A few of the larvæ seem to have passed through periods of about normal length.

D. Another small number of larvæ were also made use of to test the presence of terminal organs in the bristles. They were divided into two subdivisions as before:—

(a) Of the nine larvæ which were shorn on the evening of August 23, two had already quitted the food and were upon the roof or side. These were left in the lower of the two "opal"-covered cylinders with white-paper floors (described in III., A. and B.), the food was removed, and another larva was added which had been shorn on the

night of August 22 with those of Division A. in this series. The history of the larvæ is given below :—

| | | |
|----------------------------------|--|---|
| Aug. 23, evening (10.15 P.M.) | 2 larvæ on roof: the 3rd (last added) on floor. | |
| Aug. 24, 8.35 A.M. | The last added suspended from side : others still on roof. | Larva 1 may have been suspended about 3.30 A.M. |
| " 9.15 A.M. | Another suspended: the other has come down again and wandering. | Larva 2 suspended about 9 A.M. " 3 may have come down about 8.55 A.M. |
| " 12 noon | Last still wandering | |
| " 2.43 P.M. | It has gone up side, and is resting on it | Larva 3 may have gone up about 1.21 P.M. |
| " 5.35 P.M. | Suspended | " 3 suspended about 4 P.M. |
| " 11.30 P.M. | Suspended. | |
| Aug. 25, 8.40 A.M. | The first two larvæ have pupated some hours. | 1 and 2 may have pupated about 4 A.M. Stage III. in 1 was about 24½ hours. " in 2 was about 19 hours. |
| " 8.50 A.M. | The last larva has pupated in the last 10 minutes. | Hence Stage III occupied almost exactly 16½ hours in Larva 3. |

Two of the pupæ were near together, towards the top of the side of the cylinder farthest from the window, and 1.7 centimetres apart; the third was on the roof.

Of the 3 pupæ the 1 on the roof was a darkish (3).

Of the 2 " side 1 (the larva last added) was . . . (4), without much gold.

— 1, the last to pupate, was a very light (3).

3

(β) In the other, rather higher, cylinder two similar normal larvæ were left.

| | | |
|--------------------|---|---|
| Aug. 24, 8.35 A.M. | Both suspended | They may have suspended about 3.30 A.M. |
| " 2.55 P.M. | 1 has pupated since 2.43 P.M. | Pupation at 2.50 P.M. Hence Stage III. would be about 11 hours. |
| " 6.5 P.M. | The 2nd has pupated since 5.35 P.M. | Pupation at 6.45 P.M. Hence Stage III. would be about 15½ hours. |

The pupæ were both on the roof and 1.5 centimetres apart. The two pupæ were both very light (3).

This experiment confirms the conclusion derived from the others: that the spines do not contain any organ essential to the larval sensitiveness to colour influences. It is also seen, as in the other divisions, that the white surroundings are much less powerful than the gilt surroundings in the direction of producing the gilded appearance on the pupæ. There are also some additional data for the estimate of the duration of Stage III, and in the case of Larva 3 in the (α) subdivision the stage has been defined with practical accuracy (16½ hours). In the other larvæ there are possibilities of wide differences from the times at which it is assumed that suspension began or pupation took place, and therefore less importance is to be attached to the estimates. The preparatory period had in all cases commenced before the larvæ were periodically examined, but in Larva 3, subdivision (α), 34½ hours elapsed after the examination began. This larva was evidently in Stage II at the commencement of the

investigation, but (probably on account of disturbance) it again wandered for a short time, passing through another Stage I. of about $4\frac{1}{2}$ hours, and then again an exceedingly short Stage II. of only a little over $2\frac{1}{2}$ hours, the final stage being, perhaps, a little less than normal. These facts throw further light on the stages of certain larvæ which appear to be most abnormally short, and seem at first sight to show that it is impossible to obtain an average duration for the stages which would be of any practical value for the investigation of any particular larva. Such a conclusion might be arrived at by comparing the exceedingly divergent estimates of Stages I. and II. in Divisions B. and C. of this series, and also by comparing the estimated lengths of the total periods in these two divisions respectively. But the above-described larva shows us how after disturbance the whole preparatory period may begin again and all its stages may be passed through, but that under these circumstances the stages, and especially the two initial ones, are considerably abbreviated. It has been remarked above that the larva may even feed again after disturbance and before the recommencement of Stage I., so that the resemblance of the abbreviated period to one of normal length may be very close. And there is independent evidence for this explanation of the abnormal shortness of the stages and periods in Division C., for in the introductory sentences of Division B. it is shown that six larvæ were already in Stage II. on the morning of the 23rd, and of these only two were made use of for Division B. It is certain that many others had also entered this stage when the larvæ were shorn, and the experiments described in Division C. began in the evening of the same day, although disturbance had led the larvæ to quit the roof and in many cases to feed again both before and after they had been placed in the globes. Further data will confirm this explanation of the apparent abnormality in Division C.

Before proceeding to the next series, it will be advisable to recapitulate the results of the experiments upon the shorn larvæ, and then the subject need not be further alluded to.

| Division. | Surroundings. | Degrees of colour in pupæ of shorn larvæ | | | | | | | | Degrees of colour in pupæ of normal larvæ. | | | | | | |
|-----------|--------------------------|--|----|-----------|----|------------|---|----|------|--|----|-----------|----|------------|----|------|
| | | 1 | 2 | Dark 3 | 3 | Light 3 | 4 | 5 | | 1 | 2 | Dark 3 | 3 | Light 3 | 4 | 5 |
| A. | Gilt box. | .. | .. | .. | .. | 1 | 2 | 1 | | .. | .. | .. | .. | 1 | 3 | .. |
| C. | White "opal" globes | .. | .. | .. | 2 | 3 | 1 | .. | | .. | .. | .. | 3 | 1 | 2 | .. |
| D. | "Opal" covered cylinders | .. | .. | 1 | .. | 1 | 1 | .. | | .. | .. | .. | .. | 2 | .. | .. |
| Totals. | .. | .. | .. | 1 | 2 | 5 | 4 | 1 | = 18 | .. | .. | .. | 3 | 4 | 5 | = 12 |

The above Table shows how completely the suggestion was negatived by the experiments; it also indicates that the gilt surroundings were much more powerful

than the white, and this is further confirmed if the results of Division B. be taken into consideration. I also noted that when the pupæ produced respectively by these two surroundings were equally brilliant, and were therefore placed in the same degree of colour, those which had been influenced by the gilt surface were of a much deeper, truer, gold-colour than the others, which were often silvery-white. I had abundant opportunity of confirming this observation on subsequent occasions. The total number of pupæ obtained from this division was 31.

VI.—Another series of larvæ were also obtained from the same large nettle-bed near South Hincksey, August 22, but they were on a different part of the bed from those of the last series, and were also much less advanced. They probably constituted the whole of a very small company.

After the negative results of the experiments upon the bristles I determined to renew the blinding experiments, making use of all the precautions which had been observed in the last series.

A. In this experiment the two "opal"-covered cylinders, already frequently alluded to, were made use of. The history of the experiment is given below in a tabular form:—

| Dates, &c. | (a) 12 normal larvæ in opal-covered cylinder with white paper floor. | (g) 12 blinded larvæ in a similar cylinder |
|---|--|--|
| Aug. 25, 10.30 P.M. Aug. 26, 9.35 A.M. | Larvæ placed in cylinder | Larvæ blinded and placed in cylinder. 3 suspended; the remaining 9 reblinded; only 2 on food-plant; rest preparing for suspension. |
| " 12.10 P.M. | Only 2 on food-plant; rest getting ready for suspension | No change. |
| " 12.40 P.M. | 1 of those on food-plant is really sus- pended from it; not noticed previously | 4 suspended; only 1 on food-plant. (1 about 12.25 P.M.) |
| " 3.30 P.M. | 2 suspended altogether; but all others ready. (1 about 2 P.M.) | 7 suspended; only 1 on food-plant. (3 about 2 P.M.) |
| " 5.30 P.M. | 2 suspended altogether; but all others ready. (1 about 2 P.M.) | 7 suspended; only 1 on food plant. (3 about 2 P.M.) |
| " 7.50 P.M. | 6 suspended altogether; but all others ready. (4 about 6.40 P.M.) | 9 suspended; only 1 on food-plant. (2 about 6.40 P.M.) |
| " 9.10 P.M. | 10 suspended altogether; but all others ready. (4 about 8.30 P.M.) | 10 suspended; only 1 on food-plant. (1 about 8.30 P.M.) |
| " 10.45 P.M. | | 1 pupated, among a group on the side, quite recently; green and still very soft; 9 suspended, 1 ready for pupa- tion on floor, and 1 still feeding. |
| " 10.55 P.M. | 11 suspended (1 about 10.50 P.M.) | No change. |
| " 11.25 P.M. | No change | No change. |
| Aug. 27, 9.40 A.M. | 2 pupated an hour or two; all suspended. (Hence Stage III. about 19 hours; this must be nearly correct for 1 of the pupæ) | 8 have pupated; 1 very recently (Stage III. about 15 hours) and 2 an hour or two (Stage III. about 18 hours); the others many hours; among latter is the one lying on the floor; the last larva to feed is now ascending side. |
| " 12 NOON | 5 pupated altogether. (3 about 11 A.M., and Stage III. about 16 hours) | 9 have pupated (about 11 A.M., Stage III., 16½ hours about); the last larva still wandering. |
| " 1.15 P.M. | 10 pupated altogether (5 about 12.37 P.M., and Stage III. about 16 hours, in the case of 4 pupæ, and not very different with the 5th) | The last larva has gone up to roof. (12.37 P.M. about; Stage I. at least 3 hours.) |
| " 2.12 P.M. | 10 pupated altogether | 10 have pupated altogether. (1.45 P.M. about; Stage III. about 17½ hours.) |
| " 4.7 P.M. | 11 pupated altogether. (1 pupated about 3.10 P.M., and Stage III. about 16 hours 20 minutes) | 11 have pupated altogether. |
| " 9.30 P.M. | All 12 have now pupated | The last larva is suspended. (About 6.45 P.M.; Stage II. about 6 hours.) |
| " 10.58 P.M. | | The last larva is suspended. |
| Aug. 28, 10 A.M. | | The last larva has now pupated quite recently. (Thus Stage III. about 15 hours, and the whole period only about 24 hours since 9.40 A.M., but Stage I. may have begun much earlier.) |
| Aug. 29. Comparison of results | 8 on roof, much crowded, and of these 1 pupa was (2), darkish. 2 pupæ were dark (3) 3 " " (3) 2 " " light (3) 2 also on roof, close together, but a long distance from rest, were both light (3) 1 on side (clear glass) and isolated, very dark (3) 1 on floor attached to food, but not in shadow at all, was (4), normal, 12 with large amount of gold. | 6 very crowded on side (clear glass) towards light: these were 2 very dark (3), almost (2). 4 " (3), 1 of them rather lightish, but not a light (3). 1 on side, isolated, towards light, (4), very pink, with rather small amount of gold. 1 on floor, isolated, very light (3). 4 on roof, and isolated, were — 1 (3), 12 2 (4), golden. 1 (5), normal. |

Thus the blinded larvæ were, on the whole, more golden than the others, but of course no significance can be attributed to this. From the frequently recurring examination of the larvæ it is possible to form a very accurate estimate of the duration of Stage III., although the whole period and the other stages cannot be similarly made out from the notes. The different estimates are as follows :—

| | | | | | hrs. | min. |
|---|---|---|---|---|-----------------------------|------|
| In 2 larvæ the duration of Stage III. is estimated at | | | | | 15 | 0 |
| 7 | " | " | " | " | 16 | 0 |
| 1 | " | " | " | " | 16½ | 0 |
| 1 | " | " | " | " | 16 | 20 |
| 1 | " | " | " | " | 17½ | 0 |
| 2 | " | " | " | " | 18 | 0 |
| 2 | " | " | " | " | 19 | 0 |
| <hr/> 16 larvæ. | | | | | <hr/> Average 16 37 | |

Thus estimates were obtained for this stage in 16 larvæ out of 24, and in many cases the estimates must have been very nearly accurate. Furthermore, the extremes only differ from each other by four hours.

In addition to the above estimates, there is some insight into the other stages of a single larva in (β) subdivision, and in this instance it is shown that Stage I. must have occupied at least three hours, and Stage II. about six hours.

The experiment also shows the effect of white surroundings.

B. The globes already described in Series V. were made use of for this experiment, all the arrangements being identical, except that Nos. 5 and 11 were omitted (as they still contained larvæ of the previous series). The larvæ in the globes were alternately blinded and normal, two larvæ being placed in each globe, except two (numbered 13 and 14), for it was thought that if allowance were made for their position this number might be safely included without introducing error. Notes were not taken with sufficient frequency to render it possible to estimate the length of the stages of the preparatory period, and therefore it is unnecessary to give more than the results of the experiments. The larvæ were introduced into the globes on August 26, 10.30–11 A.M. :—

The experiment was conducted as follows:—

| Date, &c | (a) 3 larvæ transferred to gilt box for all or part of Stage III. | (β) 3 larvæ transferred to black cylinder for all or part of Stage III |
|--|---|---|
| Aug 26, 10.55 A.M. | 3 suspended larvæ transferred from food-plant to gilt box | 3 larvæ, suspended to a clear glass roof, transferred to black cylinder. |
| " 4.30 P.M. | Still suspended | Still suspended. |
| " 5.30 P.M. | 1 pupating. (Thus $6\frac{1}{2}$ hours of Stage III. were passed in the box.) | " " |
| " 7.50 P.M. | No change | " " |
| " 9.10 P.M. | Another pupated about 8.30 P.M. (Thus about $10\frac{1}{2}$ hours of Stage III. were passed in the box.) | " " |
| " 11.30 P.M. | No change | " " |
| Aug. 27, 9.40 A.M. | Last pupated many hours, about 4.35 A.M. (Thus about $17\frac{3}{4}$ hours of Stage III. were passed in the box.) | All 3 pupated many hours, about 4.35 A.M. (Thus about $17\frac{3}{4}$ hours were passed in the dark.) |
| Aug. 29. Results of comparison together and with all examined on this date. | All 3 pupæ were (3), one of them a little darkish, and none quite so light as the (3) in the dark cylinder, but very little difference between any of the (3) in gilt box or dark cylinder. | 1 of the 3 pupæ was a dark (2). 1 " " (3). 1 " " lightish (3), - rather more gold than usual. 3 |

Comparison of the dates shows that two of the larvæ in the box cannot have passed the first and presumably the sensitive part of Stage III. under the influence of gilt surroundings, and it is by no means certain that the third larva pupated at a time near the hour which is selected, for the limits were very widely separated, and the estimate therefore becomes exceedingly rough. The three larvæ in darkness all pupated in this wide interval of time, but here it becomes more probable that one or more of them passed almost the whole of Stage III. under the new conditions, and we find that one of them is very dark. It must be remembered that the latter larvæ had been previously exposed to plenty of light, and were not among dark surroundings, whereas the former were much shaded and among the dark leaves of a large amount of food-plant. The experiment, as far as it goes, certainly favours the view that the larvæ are sensitive during part of Stage III., although, standing alone, it would be totally insufficient as evidence. It is noteworthy that the larva in darkness was the only (2), except one, obtained in the whole of this series.

Thus 52 pupæ were obtained in this series.

VII.—A small number of larvæ (12), the remnant of a company, were found August 26 on a nettle-bed in Binsey churchyard, and four others, certainly belonging to the same company, were found on the church itself in the preparatory period. The negative results obtained with the shorn larvæ induced me to again attempt the blinding experiments with these larvæ also, for the results of the last series had not been obtained.

A. The 12 larvæ were made use of for this experiment, of which an account is given below :—

| Dates, &c. | (α) Blinded larvæ. | (β) Unblinded larvæ. |
|---------------------------------|--|---|
| Aug. 26, 9.35 P.M. . | 6 larvæ blinded and placed in left compartment of the gilt box already described | 6 normal larvæ placed in the right compartment. |
| „ 10.55 P.M. . | No change | 2 on roof, resting, and another wandering (Stage I. ended about 10.15 P.M.). |
| „ 11.25 P.M. . | „ | 4 on roof, resting, and 1 wandering (Stage I. of 2 more ended about 11.10 P.M.). |
| Aug. 27, 9.40 A.M. . | 5 on sides or roof, 1 feeding | 4 still on roof, resting; 1 on back, 1 on food-plant. |
| „ 12 NOON . | 4 on roof, 1 on side | 5 on roof (Stage I. of 1 more ended about 10.50 A.M.). |
| „ 1.15 P.M. . | 6 on roof (1 changed position later) . . | 2 suspended (about 12.37 P.M.), 1 on food-plant. |
| „ 4.7 P.M. . | 4 suspended (about 2.40 P.M.) | 5 suspended; 1 of them is the larva on the food-plant. (3 about 2.40 P.M.) |
| „ 5 55 P.M. . | 5 suspended (about 5 P.M.) | All 6 suspended (1 about 5 P.M.). |
| „ 10.58 P.M. . | 5 suspended | All 6 suspended |
| Aug. 28, 10 A.M. . | 5 pupated some few hours (about 7 A.M.), the 6th died. Hence Stage III. lasted about 16 hours in the case of 4 larvæ, and about 14 hours in the case of the 5th | All pupated some few hours (about 7 A.M.). Results will be given below. |
| Results, pupæ compared Aug. 29. | 1 pupa, isolated on side, was very light (3), nearly (4) 4 near together on roof, but not crowded; of these 1 external pupa is (4), normal. The other, external, is light (3). The 2 internal ones are light (3). 5 The whole period may have lasted about 30 hours, but the position of the commencement cannot be estimated with any accuracy. | 4 pupæ were arranged in an irregular (not crowded) line along one side of roof: of these 3 were (4), normal. 1 was very light (3). pink, not unusual gold. 1 was isolated on other side of roof, and was . . . (5), typical. 1 was hanging from food-plant, and was . . . (4). 6 |

Although the (α) subdivisions are not so brilliant as the (β), they are none of them dark pupæ, and there is nothing in the difference which can justify the theory that the ocelli are the larval organs influenced by the surrounding colours in the preparatory period.

The experiment also throws much light on the duration of the preparatory period and its stages. In the (α) subdivision the process of blinding probably disturbed the larvæ and prevented the normal beginning of Stage I., until it was too late to observe it, and hence the length of Stage III. is alone obtained with any degree of accuracy from these larvæ. But the normal larvæ yielded far more precise results, which are shown below :—

| | | | | | | | |
|---|--|---|---|---------------------------|--|----------------------------------|---|
| (1) The two larvae first suspended on roof | Aug. 26, 9.35 p.m. | Stage I. About 40 min., but it may have begun before 8, but not before 7 p.m., when the larvae were found | Aug. 26, 10.15 p.m., almost exact | Stage II. 14 hrs. 22 min. | Aug. 27, 12.37 p.m., almost exact | Stage III. About 18½ hrs | Aug. 28, 7 a.m., correct to 2 hrs. at the outside |
| The whole preparatory period about 33½ hrs. long: 36 hrs. if the disturbance of capture caused Stage I. to begin at once. | | | | | | | |
| (2) The second lot of two larvae to suspend from the roof | Aug. 26, 9.35 p.m. | Stage I. Certainly the whole time in the box, 1¼ hrs., and probably longer | Aug. 26, 11.10 p.m., almost exact | Stage II. 15½ hrs. . . . | Aug. 27, 2.40 p.m., must be correct to 1¼ hrs. | Stage III. About 16 hrs. 20 min. | Aug. 28, 7 a.m., correct to 2 hrs. at the outside |
| As above; the whole period 33½-36 hrs. | | | | | | | |
| (3) The fifth larva to suspend from the roof | Aug. 26, 10.15 p.m., but probably earlier | Stage I. 12½ hrs. . . . | Aug. 27, 10.50 a.m., must be correct to 1¼ hrs. | Stage II. 6 hrs, 10 min. | Aug. 27, 5 p.m., almost exact | Stage III. About 14 hrs. | Aug. 28, 7 a.m., as above |
| The whole period about 33 hrs., but probably longer. | | | | | | | |
| (4) The larva suspended on food-plant | The first stages cannot be estimated, because when a larva is on the food-plant it is assumed to be feeding. | | | | Aug. 27, 2.40 p.m., must be correct to 1¼ hrs. | Stage III. About 16 hrs. 20 min. | Aug. 28, 7 a.m., as above |

From the above Table it is possible to gain a very correct estimate of the duration of the stages and of the whole period: Stage III. is seen to be fairly constant in length, but the others very irregular. The boundaries of the different stages are fixed with great exactness in nearly all cases, owing to the number of the observations and the fact that the experiment began about two-and-a-half hours after the larvæ were taken from their food plant. I think that this Table gives the duration of the different stages more completely than they can be found in any of the other series.

B. The four larvæ found motionless, but not suspended (Stage II.), upon the porch of Binsey church were made use of in a transference experiment; the three found upon grey stone (light) being placed in black surroundings, and the one found upon the oak door (dark) being placed in white surroundings. Between their capture and subjection to these influences they were kept in ordinary chip-boxes of light wood, partially transparent. The experiment is described below:—

| Dates, &c. | (α) The larvæ found on light stone. | (β) The larvæ found on dark oak. |
|---------------------------------|---|--|
| Aug. 26, 7 P.M. . . | Found on stone; kept in chip-box till put in dark cylinder | Found on oak; kept in chip-box till put in white cylinder. |
| " 10.45 P.M. . | Placed in small cylinder covered with 1 layer of black tissue-paper and black floor | Kept in chip-box till put in white cylinder. |
| " 11.30 P.M. . | No change | Placed in small cylinder covered with white paper. |
| Aug. 27, 9.40 A.M. . | All suspended from roof | Suspended from roof. |
| " 9.30 P.M. . | " " " " " " " " " " " " | " " " " " " " " " " " " |
| " 10.58 P.M. . | 2 just pupated | " " " " " " " " " " " " |
| Aug. 28, 10 A.M. . | The last pupated very many hours . . | Pupated very many hours. |
| Results, pupæ compared Aug. 29. | Of the 3 pupæ suspended from roof, 1 was light (3). 2 were . (3). — 3 | The 1 pupa on roof was (3). |

There are not sufficient data from which to make estimates of the length of the stages.

There was extremely little difference between the pupæ, and the results agree well with those of other transference experiments, for it is probable that the pupal colour was in both (α) and (β) largely influenced by the fact that Stage II. had been passed (at any rate in part) upon surroundings which, in each case, tended in a different direction from the environment to which the pupæ were exposed in Stage III.

Thus 15 pupæ were obtained in this series.

VIII.—A. A few mature larvæ (16), the remnant of a company, were found August 28 in a field bordering the canal by Port Meadow. These were made use of in a single experiment to further confirm the negative results of former blinding experiments. In this instance the blinded and unblinded larvæ were both put in exactly similar *dark* cylinders, with black roofs and floors. This was to settle two questions: (1) whether the blinding, although insufficient to alter the influence of gilt or white surroundings, might possibly augment the influence of black surroundings; (2) whether the presence of the opaque varnish on the ocelli could possibly act itself as a stimulus, producing effects similar to the stimulus of bright surfaces.

The latter suggestion appeared to be extremely improbable, but the whole investigation seemed to be so difficult, and the results often so contrary to expectation, that it was thought better to test every possibility as it arose. And if the suggestion proved to be fruitful it was quite clear that all the results of previous experiments in which larvæ had been blinded admitted of an opposite interpretation to that which they appeared to convey, and one which would carry with it the conclusion that the ocelli really represent the terminal organ for which I was seeking. The experiment is given below:—

| Dates | (a) 8 blinded larvæ. | (b) 8 unblinded larvæ. |
|---|--|---|
| Aug. 28, 10.35 P.M. | Larvæ placed in dark cylinder. | Larvæ placed in exactly similar dark cylinder. |
| " 29, 9.45 P.M. | Nearly all suspended, or in Stage III without suspension. | All suspended, or very near it, except 3 still feeding. |
| " 30, 2.0 P.M. | 3 have pupated some little time. | 3 have pupated some little time. |
| Sept. 3. Results compared with all pupæ examined Aug. 29 and since that day. | 1 on roof was (3). 3 " " were light (3). 4 " floor " (3). — 8 Thus, altogether, 1 was (3). 7 were light (3). — 8 | 1 on roof was dark (3). 1 " " light (3). 1 " floor " dark (3). 2 " " were light (3). 1 " side was very dark (3). 1 " " " (3). 1 " " " light (3). — 8 Thus, altogether, 3 were dark (3). 1 was (3). 4 were light (3). — 8 |

Although the pupæ from the blinded larvæ were rather lighter than those from the unblinded larvæ, no one of either division was lighter than a light (3), and it is clear that there is nothing in the results which can explain the brilliant pupæ produced by blinded larvæ in white or gilt surroundings, except by the supposition that the ocelli have nothing to do with the influence. Inasmuch as the unblinded larvæ are rather

darker, it is obvious that the first of the above questions is also answered in the negative. There is every reason for the belief that these results were not influenced by the blinding one way or the other.

Thus 16 pupæ were obtained from this series.

IX.—A company of nearly mature larvæ was found August 28 on a nettle-bed (at some distance from that on which Series VIII. was found) in a field by the canal close to Port Meadow. These were kept in large, clear, glass-roofed cylinders until full-fed, and as soon as they showed signs of leaving their food the following arrangements were made :—

A. The object of this experiment was to make a decisive test of the reciprocal effects of the colours of neighbouring larvæ when much crowded. In the morning of August 31, 16 larvæ (having left their food) were selected, and 12 of them were placed in one of the smallest cylinders (8.0 centimetres high, 6.0 centimetres in internal diameter), resembling those described in Series V., B., having a similar roof, and background of glazed white paper, with about one-third of the circumference left clear, and facing a strong east light, being about one foot from the window. The floor, however, consisted of a sheet of "opal" glass. The other four larvæ were placed one in each of four other cylinders of the same kind, arranged in a precisely similar manner. All these larvæ, being much advanced, suspended themselves almost at once.

September 1, 9.30 A.M.—All the four isolated larvæ and 11 of the crowded ones had pupated, in most cases the change having taken place some hours.

September 1, 11.30 A.M.—The last larva had now pupated.

September 3.—The comparison was made between these pupæ and a very large number of those of other divisions and series, *i.e.*, with all compared August 29 and since that date.

- | | | |
|---|---|------------------------------|
| 1 | of the 4 isolated pupæ was suspended on the clear side of the cylinder, | |
| | towards light, low down, and only 8 mm. from edge of | |
| | white background | It was (4), not much gold. |
| 1 | „ 4 isolated pupæ was suspended on the side of the cylinder very | |
| | high up and just inside the edge of the background . . . | „ (5), but not extreme. |
| 1 | „ 4 isolated pupæ pupated on the white opal floor, not being | |
| | fixed to anything | „ (4), rather more gold than |
| | | the (4) above. |
| 1 | „ 4 isolated pupæ was suspended from the paper roof | „ (5), like the above (5). |

Of the 12 crowded pupæ, 7 were suspended from the roof and were all
very light (3), very uniform.

3 were suspended from the side against the white background,

of which 1 was suspended from a point 3
mm. below roof:

1 was suspended from a point 25 centimetres below roof;

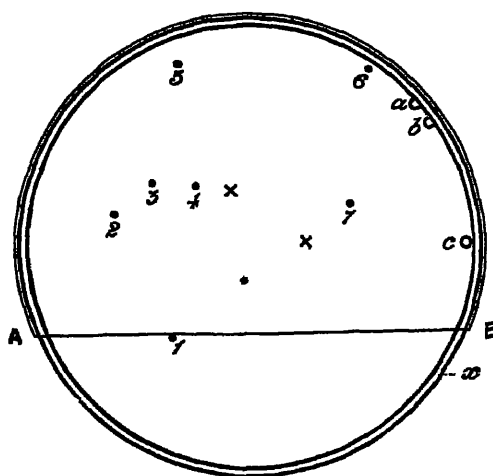
1 was suspended from a point 50 centimetres below roof:

Very light (3), very uniform, and not much gold.

Of the 12 crowded pupæ, two were on the white opal floor and not fixed to anything. They were both (4), typical.

The relative positions of these 12 larvæ are shown in Fig. 2. Natural size.

Fig. 2.



1-7 indicate the positions of the 7 pupæ suspended from the roof.

a. A pupa was attached to the side of cylinder 3 mm. below this point.

b. " " " 2.5 centimetres below this point.

| | | | | | | |
|----|---|---|---|-----|---|---|
| C. | " | " | " | 5.0 | " | " |
| G. | " | " | " | | " | " |

× × The two pupæ on the floor were probably suspended from these points in the larval state.

The inner circle represents the actual size of the roof, looked at from below.

g. The space between the two concentric circles represents the thickness of the glass sides of the cylinder.

The sides of the cylinder which bound the area below A—B were clear and uncovered by paper, and turned towards the light.

The fine line outside the cylinder above A—B represents the paper background.

Nothing could well be more conclusive than the above comparison ; of the crowded pupæ, 9 out of 12 come within a very short distance of each other, and are all (3) ; while the remaining three are more isolated in position, and the two most isolated of these are (4). Furthermore, those more completely isolated in separate cylinders are (4) and (5), not one of them reaching (3). The experiment (with others already described) seemed so conclusive that I did not consider it necessary to seek further

proof of the susceptibility of these larvæ to the dark colour of the bodies of their neighbours. This experiment is also a very good test of the influence of white surfaces upon the larvæ, and compares in an interesting way with the other divisions of this series in which gilt surroundings were used.

B. The experiments upon shorn larvæ had shown that the sensitive surface for which I was searching did not exist on the bristles, and the blinding experiments had also led to negative results. Yet the theory that the ocelli do represent the desired terminal organ affected by the coloured surroundings seemed so probable that I was desirous of obtaining other support for the experiments, which seemed at first sight to overthrow the theory so completely. These larvæ are very active, and when blinded or irritated in other ways fling their heads about in the most violent manner, and I feared that the varnish might in this way have been accidentally knocked off one or more of the six ocelli on one or both sides. In the most careful experiments the application of varnish had been repeated, so that three coats had been given in many instances, but the larvæ had been violent whenever the process was repeated. Again, it seemed possible that if the varnish had not been removed it might have been made thin and partially transparent over the ocelli, most of which project considerably. In spite of these arguments I did not see how my repeated and careful application of varnish could have failed in all cases; I did not expect to find the blinded larvæ as dark as those exposed to black surfaces, but I did expect that they would not be equally brilliant with normal larvæ when both were exposed to white or gilt surfaces, that is, if the ocelli represented the desired terminal organs. But the above considerations made it very desirable to test the ocelli in some other way. I had long thought of another experiment, in which the head and the body of a larva were to be exposed respectively to two different colours, producing the most opposite effects, but the difficulty was in the mode of application. In fact, this difficulty seemed insuperable for Stages I. and II. of the preparatory period, but perhaps might be overcome with a little contrivance for Stage III., in which the larva rests suspended. It was, however, first necessary to decide whether the larva is sensitive to colour during this stage. Previous experiments (V., B.; VI., C.; VII., B.) had not thrown much light on the question, but the numbers employed had been insufficient. I therefore determined to devote the majority of the larvæ of this series to the settlement of this question. It seemed that a decisive answer could be best obtained by the transference of larvæ in sufficient numbers at the beginning of Stage III. from a colour to which they had been up to that time exposed into one which tended to produce the most opposite effect. Quite apart from the chief object to be served by the experiment, *i.e.*, the possible introduction of further experiments, the results would be most interesting on their own account in the further light which would be thrown upon the preparatory period. At 2.30 P.M., August 31, it was seen that the larvæ were nearly all mature, and were beginning to collect on the sides and roof

of the large cylinders in which the stock was kept. At this time, therefore, the arrangements for transference were made. Between 2.30 and 3.15 P.M. 17 larvæ were placed in a large cylinder covered with one layer of black tissue-paper, and with a black floor, the whole being covered with two black opaque mats and placed on the floor in shadow (this is II. of the Table below); 12 larvæ were placed in each compartment of the gilt box, arranged as usual (IV. below), and 12 more in another gilt receptacle (also IV. below), of the following construction. I covered a cylinder (1.86 decimetre in internal diameter and 8 centimetres in height) with gilt paper, and covered it with a gilt-paper roof. The cylinder was placed on its side, with its open end facing the light, and therefore a segment of the side formed the roof, while the back was formed by gilt paper (which would have been the roof in the other and usual position of the cylinders used in these experiments). The open front was closed by a plate of clear glass. This gilt cylinder was made use of in many other experiments, and will be always spoken of shortly as the "gilt drum." Another large cylinder was covered with two layers of black tissue-paper, with a black floor, and placed in shadow (I. below), and into this many of the larvæ from the "gilt drum" and box were transferred at the beginning of Stage III. When transferred, the larvæ were in nearly all cases pinned by the boss of silk, to which the posterior claspers adhered. The experiments, together with their results, are given in a tabulated form below:—

| | I.—Transferred from gold into black for Stage III. | II.—In black for the whole period. | III.—Transferred from black into gold for Stage III. | IV.—In gold for the whole period. |
|-----------------------------|---|--|--|--|
| Aug. 31. 9.30-10.15 P.M. | 9 transferred from gold drum 2 suspended from gold drum 4 suspended from gold box 3 near suspension from drum | 8 larvae feeding 9 others transferred to gold after this date | 2 transferred from black into gold drum; both suspended | 6 larvae feeding in gold drum 6 others transferred to black at or after this date 14 larvae feeding in gold box 10 others transferred to black at or after this date. |
| Sept. 1. 9.30-10.45 A.M. | 1 pupated some hours Rest suspended (1 only transferred for few hours) No further change | No change | At 9.30 A.M. 1 was seen to pupate (Transferred for about 11½ hours) The 2nd has just pupated (Transferred for about 14 hours) | 1 pupated some hours No change No change |
| 11.30 A.M. | | " | " | 3 pupated |
| 12.25 P.M. | 2 pupated, 1 having fallen off roof (2 transferred for about 14½ hrs) No change | " | " | No change 2 pupated 1 pupating |
| 12.55 P.M. 2.15 P.M. | 3 pupated (3 transferred for about 16 hours) No change | " | " | No change 2 pupated 1 pupating |
| 3 P.M. 11.50 P.M. | 2 had pupated some hours, and 1 was dead (2 transferred about 21¼ hours; it cannot have been for a less period than 17 hours) | 4 suspended and (about 7.30 P.M.) 1 on roof Others feeding, except one or two preparing for pupation on the floor | 5 pupated an hour or two (Transferred for about 12 hours) | 1 pupating 4 pupated, 1 of them recently. |
| Sept. 2. 7.45 A.M. | | No change | 2 last had pupated (Transferred for longer than the above, but it is impossible to make a correct estimate) | All pupated, except 2 suspended. |
| 6 P.M. | | " | " | No change |

| | I.—Transferred from gold into black for Stage III. | II.—In black for the whole period. | III.—Transferred from black into gold for Stage III | IV.—In gold for the whole period. |
|---|---|---|--|---|
| Sept. 2. 7.30 P.M. | .. | 5 pupated (about 6.45 P.M.; hence Stage III. about 24 hours duration) 2 suspended 1 feeding No change, ditto 1 more had pupated 1 died 1 pupated much later | .. | .. |
| 10.5 P.M. Sept. 3. 9.50 A.M. | .. | .. | .. | 1 pupating 1 pupated many hours |
| Result of colour-comparisons :— Sept. 3, except for a few which had not darkened sufficiently; these were compared later | 1 had died 4 suspended from roof were all (4), 1 with little gold 4 on floor, of which 2 were light (3), 2 were (4), 1 with little gold 9 | All remained suspended to roof. 4 were light (4), 3 were (4), 1 with typical gold, 2 with very little gold for (4) 7 | 1 pupa pinned on gold back is (3) 1 fastened on to roof to the silk boss of a pupa (removed) is (3) No extra gold on either 2 | 1 on floor (4) (gold) is a Of 5 on roof (but not crowded), 1 is light (3), 2 are (4), 2 are (5) All with the gold usual in these degrees, except the (5), which have rather less 6 |
| | Probably more effect would have been produced if the larvae had been shifted at the very beginning of Stage III., but the above estimates show that this can only have happened in very few cases | | | 2 close together on roof of one compartment were a (4) and a (5) normal, 1 near them was (4), 3 others, more isolated, were— 2 . . . (4), 1 . . . (5) In other compartment— 2 fell on to brown-paper floor, and were both very light (3) 2 isolated on roof and back were (5) and near together (not crowded) were—the 2 middle ones both light (3), the 2 external ones both (5). 14 |

The results of this Table will be most clearly expressed in the following analysis :—

| The degrees of colour from (1), darkest and least golden, to (5), lightest and most golden. | (1) | (2) | Dark (3) | (4) | Light (3) | (4) | (5) | |
|--|-----|-----|-------------|-----|--------------|-----|-----|------|
| II. In black for the whole period | .. | 1 | .. | 5 | .. | 1 | .. | = 7 |
| I. Transferred from gold into black, for Stage III. | .. | .. | .. | .. | 6 | 9 | .. | = 15 |
| III. " " black into gold " " | .. | .. | .. | 1 | 5 | 3 | .. | = 9 |
| IV. In gold for the whole period | .. | .. | .. | .. | 5 | 7 | 8 | = 20 |
| | | | | | | | | 51 |

Such an analysis speaks for itself ; it is quite clear that the pupæ as a whole tend strongly towards the lighter forms, but it is equally clear that Numbers I. and III. are intermediate between the two extremes II. and IV. In the considerably lighter results of the former (I.) we probably see the proof that in the shorter stage, II., the influences are really more potent than in III., which is considerably longer. Similarly Stage II. was passed in black surroundings in the case of III., and its results are darker than those of I. In fact, in both I. and III. the colour in which Stage II. was passed predominates in the result, or rather it is more accurate to say that the gilt surroundings produce more effect in Stages I. and II. than in Stage III. alone. It is also probable that the earliest, most sensitive, part of Stage III. had already elapsed in most cases before transference took place. But further experiments will show the inferior susceptibility of Stage III. under the most favourable conditions, and indeed such a result might have been expected, for, although this stage is, as a rule, so much longer than any other, the larval sensory surfaces are probably only in a condition to be influenced in its most early part, for very rapid changes of pupal construction and shape are going on beneath the surface. These would seem to preclude the possibility of an external shell, shortly to be cast off, having any important physiological relation with the organism beneath. But in Stage II. the larva retains its shape, and the whole of its surface is in close relation with the colour into correspondence with which the pupal tints will afterwards deepen. On the other hand, the posterior part of the suspended larva is alone in close proximity with the surface to which it is fixed. But nevertheless the comparison of III. with IV., and I. with II., shows equally clearly that the larva is susceptible, and to a considerable extent, during Stage III., although the susceptibility is probably confined to the first part of it. Future experiments will supply the means for testing the sensitiveness of the different parts of this stage. This experiment showed that an investigation by the use of conflicting colours applied to Stage III. could be undertaken with, at any rate, a fair prospect of success. After all the other experiments it is almost unnecessary to point out how entirely the former theory of pupal as opposed to larval sensitiveness is broken down by the analysis given above.

C. Another small transference experiment was made with the three larvæ found suspended to the food-plant at 2.30 P.M., August 31, when most of the larvæ were re-arranged. These three were then transferred to the gilt box, two being pinned up against the side and one on the roof.

September 1, 9.30 A.M. All had now pupated; the one on the roof last night, and the two on the side some hours.

September 3, morning. Comparison produced these results:—

| |
|--|
| Of the 3 pupæ, 1 which pupated evening of Aug. 31 (on roof) was (1), very black pigment. |
| „ 3 „ 2 „ later (on side) . . . 1 „ (4), less gold than usual. |
| 1 „ (5), normal gold. |

When these three suspended larvæ were transferred August 31, one pupa was also found suspended from the food-plant: it was a light (3).

These results harmonise well with the more complete transference experiments in Division B. The dark transferred pupa was only exposed in the larval state for the last few hours of Stage III., and the resulting colour shows that it could not have been influenced at all. It was evidently an exceptionally dark form for this series. The others were exposed for probably the whole of Stage III. and were much affected, one of them being brighter than any of the transferred pupæ in Division B. The latter result must be also partially due to the fact that the larvæ had been previously exposed to surroundings which were far less dark than those made use of in B.

D. Another experiment was made upon six mature larvæ, which were placed, August 31, 2.30 P.M., in a small cylinder resembling those described in Series V., B., covered with two layers of black tissue-paper, but lined with gilt paper. The larvæ had the opportunity of being influenced by the gold, illuminated by daylight, for a few seconds before the cylinder was placed on a black floor, with another double-layered black cylinder over it, in a dark cupboard.

September 2, 7.30 P.M. The cylinder was not touched till this date, but I had estimated that they would have pupated long before this time, and therefore the cylinder was now removed, and it was found that all six had pupated on the roof and had taken their final colour.

September 3, morning. The pupæ were compared with all the others, giving these results:—

| |
|---|
| Of the 6 pupæ, 3 were light (3). |
| 1 was . . (4), but with little gold. |
| 1 „ . . (5), not much gold for this degree. |
| — |
| 5 |

The colour of the 6th pupa was accidentally omitted from the notes.

It is possible that these results were influenced by the few seconds of exposure to the gilt in a bright light, or by the exceedingly small amount of light which may have penetrated. But, on the other hand, the whole series tends strongly towards the

lighter degrees, and the single (5) may have been merely an individual which tended especially strongly in this direction. The results nevertheless suggested a course of experiments which would be likely to give very interesting results, but which I had not time or material to undertake. The suggested experiments were to ascertain the effects produced by some powerfully acting colour (as gold) under different conditions of illumination. On theoretical grounds it is unlikely that the ratio between the two will prove to be direct, but it is to be expected that diminution in illumination will not be attended by a corresponding diminution in effect.

The number of pupæ obtained in Series IX. was 76, and it is therefore probable that the whole of a rather small company was obtained.

X.—A. The remnant of a company was found August 29 near South Hincksey. It was arranged to make use of the few larvæ (eight only) in an experiment to further test the effect of transference during Stage III. into a colour with an influence opposite to that of the colour to which the larva had been previously exposed.

The experiment was conducted as follows. The gilt box and drum and a black cylinder were made use of:—

| | Compartment of gilt box. | Gilt drum. | Black cylinder, with black roof and floor. |
|---|---|---|--|
| Aug. 29, 7 P.M. | 8 larvæ put in compartment, having been captured about 5 P.M. | | |
| " 8.30 P.M. | 4 moved into gilt drum | 4 larvæ introduced. | |
| Aug. 30, morning, early | Food hardly touched, larvæ crawling up sides or motionless on roof | Food hardly touched, larvæ crawling up sides or motionless on roof. | |
| " 11.30 A.M. | | | |
| " 1.20 P.M. | 1 suspended | 1 suspended." | |
| " 1.50 P.M. | " | Another suspended, and at once moved into black surroundings | 1 larva only just suspended, moved from gilt drum. |
| " afternoon | " | Another suspended and similarly transferred | A second larva introduced. |
| " evening | 2 suspended altogether. | | |
| Aug. 31, 9 A.M. | 2 pupated some time, 1 suspended | 2 pupated some time, the time of suspension of 1 not noted | 2 pupated: not long, as they have not darkened completely yet. |
| " 9 P.M. | The last pupated recently. 1 larva died. | | |
| Results: Sept. 1. Compared with all examined on this day, and 45 examined Aug. 29 | The 3 pupæ were all (4), not much gold, but quite light-coloured, 1 rather darker than any of the 7 | The 2 pupæ were both (4), not much gold, but quite light | The 2 pupæ were both typical (4), with typical gold; thus the most golden of the 7, but all are equally light, except 1 of those in compartment of gilt box. |

These results are very curious and to some extent accidental, as is proved by comparison with the results of similar, but larger, transference experiments. Such a comparison shows that there is obviously no significance in the gilt surroundings producing *more* effect when acting only in the Stages I. and II. of the preparatory period than when acting in these and in Stage III. also. The highly-marked effects seen in the two transferred larvæ are doubtless somewhat abnormally extreme results of the very powerful influence of gilt surroundings working during a time of very high larval susceptibility, *i.e.*, Stage II.

Thus seven pupæ were obtained from this series.

XI.—I was very anxious to obtain some wild pupæ in order to compare their colours with those of the pupæ which had been the subjects of experiment. From what I remembered of observations in former years, I felt assured that the common degree of colour was that represented by (3), but that (2) and (1) were not uncommon, while the highly gilded forms (4) and (5) were almost unknown, except in pupæ which contain *Ichneumon* larvæ. This exception is, however, obviously abnormal, and it will be alluded to below. On August 31 I found 15 pupæ of *V. urticæ* on a smooth stone wall, with an east aspect, in Oxford. Inasmuch as the pupæ occurred near together, along one continuous extent of wall, and were about the same age, there is little doubt that they were all produced from the larvæ of a single company. Two-thirds of the pupæ were found under the projecting coping. The colour of the stone was grey from the growth of lichens and from the darkening due to soot, &c. Under the coping the colour was especially dark, and was further intensified by the shadow. The pupæ were not sufficiently crowded for their colour to have been mutually affected.

The pupæ were most carefully compared with 45 others examined August 29, and with all examined September 1, and the results were as follows:—

| | |
|--|--|
| Of the 15 pupæ, 4 were . . (1), with no gold at all. | |
| 1 was . . (2), " " | |
| 7 were dark (3) } | Only the minutest spot of gold to be seen on careful examination |
| 3 " . . (3) } | in 2 of these: none in the others. |
| <hr/> 15 | |

The gold of the two pupæ was in the position in which it always occurs if present at all, *i.e.*, round the base of the small lateral tubercles on the first and second abdominal segments. There was a strongly marked deep reddish tint in the three (3), and to a less extent in five out of the seven dark (3) and in one of the (1). This colour was much deeper than the pink tint so often mentioned in the pupæ of my experiments. It was, however, similar to the red mentioned in Series XII., B. and D., although it was much deeper than in the latter. These 15 pupæ were so different

from all the others that it was very hard to classify them according to the same standard, but I am quite sure that there has been no error in the direction of making them appear too dark; if there has been any mistake, it has been in the other direction.

Thus 15 pupæ were obtained in this series.

XII.—A large company of nearly mature larvæ was found August 31 on a nettle-bed near South Hincksey, different from that on which the other series had been found. In a few days the larvæ became mature, almost simultaneously, and the following experiments were made:—

A. The object of this experiment was to ascertain whether a black surface in a powerful light has a different effect from that of complete darkness.

(a) 13 larvæ were placed in a cylinder covered with two layers of black tissue-paper, and a similar roof; a black floor was added, and the whole was placed in shadow. The experiment and its results are given below:—

| | |
|---|--|
| Sept. 3, 10.15 A.M.—1.43 P.M. | The 13 larvæ were introduced into the cylinder at these hours, and at various times between them. |
| " 11.10 P.M. . . . | 5 suspended; all the others except 1 are resting on roof. |
| Sept. 4, 1.15 P.M. . . . | All suspended except 1, and it is in Stage III. on the floor, unfixed. |
| " 6.30 P.M. . . . | 2 pupated some little time. (If pupation be estimated at 5 P.M. and suspension at 11 P.M., Sept. 3, Stage III. would be 18 hours; but it was probably longer.) |
| " 9 P.M. . . . | 2 pupated. (If pupation be estimated at 7.45 P.M. and suspension at 11 P.M., Sept. 3, Stage III. would be 20½ hours; but it was probably longer.) |
| " 12, MIDNIGHT . . . | 3 pupated. (If pupation be estimated at 10.30 P.M. and suspension at 11 P.M. for 1 of the larvæ, Stage III. would be 23½ hours; but it was probably longer.) |
| Sept. 5, 10 A.M. . . . | All have pupated some hours. (If pupation took place at 5 A.M. and suspension at 6 A.M., Sept. 4, Stage III. would be 23 hours; but the estimate is very rough.) |
| Results of comparison, Sept. 7 (with all others mentioned as compared on this day). | <p>1 pupa on floor was (3), lightish, but not a light (3). 1 " on side was light (3), with little gold, but more than any other of the 13.</p> <p>Of the 11 pupæ scattered over the roof, but not crowded— 2 were (1), very black indeed. 2 " (2). 4 " dark (3). 2 " (3). 1 was light (3).</p> <p>13</p> |

(β) 14 larvæ were placed in a similar shallow black cylinder, which was then made to rest on its side with the open end covered with a clear glass plate, directed towards a strong east light, and close to the window. Hence the roof of the cylinder was a segment of the side (in the other position), and the ordinary roof of black tissue-paper formed the back. The experiment and its results are given below:—

| | |
|---|---|
| Sept. 3, 10.15 A.M.—3 P.M. . . | The 14 larvæ were introduced into the cylinder at these hours, and at various times between them. |
| „ 3 P.M. | 1 suspended. (About 12.23 P.M.) |
| „ 3.45 P.M. | 1 suspended. (About 3.22 P.M.) |
| „ 6.15 P.M. | 1 suspended (about 5 P.M.), but all the rest on the top. |
| „ 11 P.M. | 2 suspended (about 8.23 P.M.), but many others with the boss of silk spun and ready for suspension. |
| Sept. 4, 9.25 A.M. | All 14 suspended, but no pupation yet. |
| „ 1 P.M. | 2 pupated an hour or so. (If pupation be fixed at 12 A.M., the duration of Stage III. would be 23 hours 37 minutes and 20 hours 38 minutes respectively.) |
| „ 6.30 P.M. | 7 pupated at various times since the hour last noted. (If 3.45 P.M. be estimated as the time of pupation, Stage III. would be 22½ hours in one case and rather over 19 hours in two others.) |
| „ 8.30 P.M. | 4 pupated; 1 quite recently. |
| „ 12 MIDNIGHT | 1 has now pupated; the last. |
| Results of comparison, Sept. 7 (with all others mentioned as compared on this day). | <p>All 14 were hanging together from the tissue-paper top of the cylinder, i.e., that part of its side which was uppermost, and which was lined internally with black tissue-paper. There was hardly any gold, or none at all, on these pupæ.</p> <p>Of the 14 pupæ—</p> <p>3 were rather outlying on one side, and of these 2 were (3). 1 was dark (3).</p> <p>1 was rather outlying on the other side, and was (3). 10 formed a central group, of which 3 were (2). 2 „ dark (3). 5 „ (3). — 14</p> |

Analysing the results of the two experiments, we find the following :—

| Degrees of colour: (1), darkest and least golden, (5), lightest and most golden. | (1) | (2) | Dark (3). | (3) | Light (3). | (4) | (5) |
|---|-----|-----|--------------|-----|---------------|-----|-----|
| (α) Black surroundings in the dark . . . | 2 | 2 | 4 | 3 | 2 | . | .. |
| (β) Black surroundings in strong light . . | .. | 3 | 3 | 8 | .. | | .. |

Upon the whole, the results are not widely different, and, as further throwing light on the comparison, I find a note that the light (3) on the side of the cylinder in (α) subdivision is much more golden than any pupæ in the (β) subdivision, which, as a whole, were rather distinguished from the others by the extreme absence of gold. It is to be noted that the larvæ of (β) did not suspend themselves as soon as the others, and therefore passed a longer part of the preparatory period in their cylinders.

The result of the experiment was very satisfactory in its bearing upon the proposed conflicting colour experiments, for in these I could not well place part of a larva in complete darkness, while it would be comparatively easy to surround it by black

surfaces, which this experiment shows to be decidedly efficacious, although not quite equal to the same surface in darkness.

B. The object of this experiment was to test the effect of gilt surroundings on larvæ when they were exposed to its influence for the whole of the preparatory period as compared with exposure during Stage III. only. This was accomplished by placing eight mature larvæ with food in one compartment of the gilt box, and transferring recently suspended larvæ to the same compartment, where they were pinned against the gilt side. Both subdivisions were taken from the stock under clear glass in large cylinders. The experiment was conducted as follows:—

| Dates, &c | (a) 8 larvæ exposed to gilt surroundings for preparatory period | (β) 8 larvæ exposed to gilt surroundings for Stage III |
|----------------------------|--|--|
| Sept. 3, MORNING | .. | 2 suspended larvæ pinned in compartment within few minutes of beginning of suspension. |
| " 11 27 A.M. | 8 larvæ placed in the compartment | No change. |
| " 3 P.M. . . | 1 suspended, 3 resting on roof, 1 wandering, 3 feeding | " |
| " 6.15 P.M. . | No change | " |
| " 11 P.M. . . | 3 suspended, 4 resting on roof . . | " |
| Sept. 4, 8.35 A.M. . | 4 " 3 " " . . | 2 have pupated 2 or 3 hours. (Hence about 20 hours were passed in the compartment.) |
| " 1 P.M. . . | 5 " 2 " " . . | .. |
| " 1.10 P.M. . | " " " " . . | .. Another suspended larva added. |
| " 1.35 P.M. . | 1 has just pupated. (Hence Stage III. is probably very nearly 24 hours long.) | . No change. |
| " 4.15 P.M. . | 1 has just pupated. (Hence Stage III. probably about 19-20 hours.) | .. " |
| " 8.50 P.M. . | .. | .. It has just pupated. |
| Results: compared Sept. 7. | 1 pupa suspended to side, near the pinned ones, was (4), with normal gold. Of 5 pupæ suspended on roof, 1 was very dark (3). 2 were light (3) with little gold. 2 were (4), 1 with normal gold, and 1 with little. There is no note of pupations later than the above 2. 2 larvæ died. | Both pupæ were light (3), 1 of them very red and black in lower part of abdomen (? diseased). These larvæ must have spent very nearly all Stage III. in the box, and seem to have been somewhat affected. It is a dark (3). Red in lower part of abdomen. This larva, having been only about 7½ hours in the box, appears to have been unaffected. |

The results are analysed in the following Table :—

| | (1) | (2) | Dark (8) | (3) | Light (8) | (4) | (5) |
|--|-----|-----|-------------|-----|--------------|-----|-----|
| 6 pupæ exposed to gilt surroundings for the whole period | .. | .. | 1 | .. | 2 | 3 | .. |
| 2 pupæ exposed to gilt surroundings for the whole period of Stage III. | .. | | .. | | 2 | .. | .. |
| 1 pupa exposed to gilt surroundings for the last part of Stage III. | . | . | 1 | . | .. | .. | .. |

It is probable that the above results indicate, on the whole, the relative susceptibility of Stage III. to the whole period, although, at the same time, the single dark pupa in the first line shows that we must be prepared for exceptions, and that it is necessary to make use of large numbers of larvæ in order to obtain a sufficiently accurate result.

C. It seemed that interesting results might be obtained by exposing a few larvæ during Stage III. to a very powerful direct light, and yet without any coloured background, at a distance which could affect them. If negative results were obtained, they would serve as a confirmation of conclusions rendered probable by other experiments, viz., that the effective influence is due to the presence or absence of reflected light, and its quality, if present, but seldom or never under natural conditions, to direct light falling upon the larvæ. In the following experiments I proposed to place the larvæ under conditions in which they could only be illuminated by direct light. This object was achieved by pinning the suspended larvæ to the central vertical bar of a large east window, the pin being thrust through the boss of silk so far that the larvæ hung suspended from the head of the pin which projected from the side of the bar, so that the whole length of the pin intervened between the larva and the bar (painted stone-colour). Very long pins were made use of in the experiment. The suspended larvæ were taken from the main stock in the cylinders, where they were found fixed to the food-plant. The following Table explains the manner in which the experiment was conducted :—

| | | | | |
|-------------------------------------|---|--|--------------|--|
| Sept. 3, 11.30 A.M. . | 1 pinned up. | 1 pinned up. | | |
| " 1.5 P.M. . | " | " | 2 pinned up. | |
| " 4.45 P.M. . | " | " | 1 fell off. | 4 pinned up. |
| " 6.15 P.M. . | " | " | " | 2 pupated; some hours. |
| Sept. 4, 8.35 A.M. . | " | " | " | 1 pupating. |
| " 11.0 A.M. . | Pupated some little time. | " | 1 dead | |
| " 11.30 A.M. . | " | Pupating | .. | No change. |
| " 1 P.M. . | .. | .. | .. | 1 pupating. |
| Results:— Compared Sept. 7. . | Pupa was (3), normal gold. Thus Stage III. very long, apparently about 22–23 hours. | Pupa was (5), golden, but not extreme for this degree. Thus Stage III. also very long, and at least 22½ hours. | | 2 first to pupate were dark (3) with rather unusually bright gold for this degree, but the normal amount. 2 next light (3), normal gold. The first two only passed about 8–9 hours in the light. The 3rd 16½ hours, and the 4th 18½ hours. |

The results of the experiment are certainly surprising, for I did not expect to find any indications of influence, and at the time of the experiment I did not think that there were any. I was then taking notes and making experiments every hour of the day, and had not time or opportunity to make allowance for the periods during which the larvæ had been under any influence. However, when the notes are worked out in the above tabular form, there seems to be much reason for thinking that some considerable effect was produced. The only two dark (3) among the pupæ were those which were pinned up in the window long after the susceptible part of Stage III. had already passed, and the others, which were exposed for practically the whole of the stage, are certainly lighter than the normal forms. Had I realised these results at the time, I should have made other and much larger experiments, avoiding the source of error introduced by the proximity of the vertical light-coloured bar by suspending the pupæ from fine threads. I hope to make such an experiment in the next season. The results are all the more remarkable because in Division A. it was shown that the powerful direct light had but little influence in opposition to the black surroundings.

D. The rest of the larvæ were almost entirely made use of in the conflicting colour experiments, towards which many other experiments had been leading. Two frames were made on precisely the same principle as a larger one, which will be described and illustrated in the next series, and therefore I need only give a mere outline of the

construction in this place. The bottoms of two flat wooden trays were, in each case, covered with black and gilt paper, the different colours meeting along a line which ran across the tray, and along which a shelf was fixed covered with gilt paper towards the gilt side of the tray, and black towards the black side. The shelves close to the tray bottom were perforated with holes separated by equal distances, and the size of each hole was such as to easily admit the body of a larva, with its spines, but sufficiently small to prevent the occurrence of any considerable space between the edge of the aperture and the larval body. In fact, such space as existed was much obscured by the larval spines. The trays were placed vertically, with the coloured surfaces facing a strong east light, and close to the window, so that the shelves projected horizontally; but the black surface was uppermost in one tray, and the gilt surface in the other.

Whenever suspended larvæ were found among the food-plant, &c., of the cylinder containing the stock they were pinned on to the part of the trays covered with the upper colour, in such a position over the holes that the head and thoracic segments, and generally the first and second abdominal segments, of each larva passed through a hole into the colour beneath, which tended to produce opposite results. This anterior part of the body being always strongly curved in Stage III., the head with any sensory organs upon it was brought close up to the under-side of the shelf, and thus there was no chance of its being influenced in any way by the colour above the shelf (which was of considerable depth). Thus rather more than half the total skin area was exposed to the upper colour, while rather less than half, together with the head, was exposed to the under colour. If the head contained the sensitive surface which was being sought for, we should expect that the pupa would be coloured according to the influence—already known and gauged—of the lower colour; but if, on the other hand, the whole larval surface was susceptible to colour we should expect that the results would oscillate sometimes on one side and sometimes on the other, but that, on the whole, the preponderance would be in the direction of the tendencies produced by the upper colour, inasmuch as there was a rather greater surface of skin above the shelf than below it. Beneath the shelf other larvæ were fixed upon the lower colour only in the case of each frame, in order to form a comparison-experiment. In the tabulated account of the experiment, given below, P stands for pupation, and F for placing a larva on the frame; while r indicates that pupation took place recently, or that the larva was placed on the frame directly after suspension.

| Date, &c. | A. The head and anterior part of larvae in gilt surroundings; the larger posterior part in black surroundings | | | | | | | | | | | B. The larvae in gilt surroundings. | | | | | |
|---|---|-----|-----|----------|-----|-----------|-----|-----|-----|------------------------------------|--------------------------------------|-------------------------------------|-----|-----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 1 | 2 | 3 | 4 | 5 | 6 |
| Sept. 4, 11 A.M. to 12 noon | 11 larvae put on the frame; all known about suspension is that it had not begun 11.15 P.M., Sept. 3. | | | | | | | | | | | | | | | | |
| Sept. 4, 2.30 P.M. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| " 4.30 P.M. | .. | .. | .. | .. | .. | P r | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| " 6.30 P.M. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| " 8.50 P.M. | .. | .. | .. | .. | .. | .. | .. | P r | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Pupation quite recent in nearly all these cases | | | | | | | | | | | | | | | | | |
| 12 minutes | P | P | .. | P | P | .. | P | .. | P | P | P | P | P | P | P | P | P |
| Sept. 5, 10 A.M. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| " 2.30 P.M. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Results of comparison Sept. 7 | (3) | (3) | (3) | Dark (3) | (3) | Light (3) | (3) | (3) | (3) | Very light (3), good deal of gold. | Very dull (3), and with little gold. | (4) | (5) | (4) | (4) | (4) | (4) |
| Very dull, with little gold. A slight reddish tinge on posterior part of abdomen in Nos. 2, 5, 7, 8, and 10. | | | | | | | | | | | | | | | | | |

| Dates, &c. | C. The head and anterior part of larve in black surroundings; the larger posterior part in gilt surroundings. | | | | | | | | | | | | | D. The larve in black surroundings. | | | | | |
|---|---|-----|----------------------------|-----------|-----------|--------------------------------|-----------|----------|----------|----------|----------|-----|----------|-------------------------------------|-----|-----------|-----------|----------------|-----------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 1 | 2 | 3 | 4 | 5 | 6 |
| Sept. 4, 11 A.M. to 12 noon | 18 larve put on the frame. all known about suspension is that it had not begun 11 15 P.M., Sept. 8. | | | | | | | | | | | | | F | | | | | |
| Sept. 4, 1.15 P.M. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | Pr | Pr | Pr | Pr | Pr |
| " " 8.50 P.M. | .. | .. | .. | .. | .. | .. | .. | .. | Pr | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| " " 6.20 P.M. | .. | .. | .. | .. | .. | Pr | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| " " 6.30 P.M. | .. | .. | .. | .. | .. | Pupa fell down injured. | .. | Pr | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| " " 8.50 P.M. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Pupation quite recent in nearly all these cases | | | | | | | | | | | | | | | | | | | |
| 12 MIDNIGHT | .. | P | P | P | .. | .. | P | .. | .. | P | P | P | P | P | P | Pr | P | P | P |
| Sept. 5, 10 A.M. | P | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| " " 2.30 P.M. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Results of comparison Sept. 7 | Light (3) | (2) | deformed, but probably (3) | light (3) | light (3) | injured, but probably dark (3) | light (3) | dark (3) | dark (3) | dark (3) | dark (3) | (3) | dark (3) | very light (3) | (4) | light (3) | light (3) | very light (3) | light (3) |
| A reddish tinge on posterior part of abdomen, pronounced Nos. 8, 9, 10, 11, and 12, and slight in Nos. 4 and 5. | | | | | | | | | | | | | | | | | | | |

An examination of the dates at which pupation took place in A. and C. unfortunately shows that in nearly all cases an important part of Stage III. had elapsed before the larvæ were pinned on the frames. It is quite clear that A., 6, 8, and C., 6, 8, 9, cannot have been influenced after the transference, and there were probably only two larvæ in A. (3, 4), and two in C. (1, 5), which may be considered to have satisfied the conditions of the experiment. The larvæ which were found to have pupated by 12 P.M., September 4, cannot have passed as much as 13 hours on the frame, and most of them must have had a very much shorter period of time. The larvæ of B. and D., on the other hand, seem to have passed the whole of Stage III. on the frame, and one of the most curious and exceptional things in all the experiments recorded in this paper is the fact that the larvæ of D., surrounded entirely by black for the whole stage, should be lighter than those of A. or D., which were also partially exposed to gilt, and had been on the frame for a much shorter time in nearly all cases. In other respects, however, an analysis of the few results in which the influences had worked for an adequate period of time is very satisfactory.

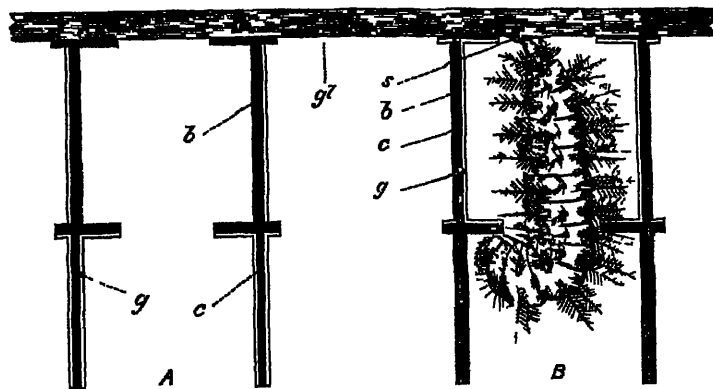
| Degrees of colour. | (1) | (2) | Very dark (8) | Dark (8) | (8) | Light (8) | Very light (8) | (4) | (5) | |
|---|-----|-----|---------------|----------|-----|-----------|----------------|-----|-----|------|
| A. Larger skin area in black, smaller in gilt for Stage III. | .. | .. | .. | 1 | 1 | .. | . | .. | .. | = 2 |
| C. Larger skin area in gilt, smaller in black for Stage III. | .. | .. | .. | .. | .. | 2 | .. | .. | .. | = 2 |
| B. Entirely exposed to gilt for Stage III. . . . | . | .. | .. | .. | .. | . | . | 5 | 1 | = 6 |
| D. Entirely exposed to black for Stage III. . . . | .. | .. | .. | . | . | 3 | 2 | 1 | .. | = 6 |
| It is interesting to analyse in a similar manner the results of A. and C., including those which had pupated at 12 P.M., September 4. | | | | | | | | | | |
| A. | .. | 1 | .. | 1 | 7 | .. | 1 | .. | .. | = 9 |
| C. | .. | 1 | .. | 2 | 8 | 4 | .. | .. | .. | = 10 |

The comparison between A. and C. in the upper of the above Tables confirms the results of the previous blinding experiments, showing that no sense-organ in the head can possess the property of being influenced by light in such a manner as to direct the formation of colour in the pupa. Conversely, the results strongly favour the view, and, in fact, confirmed as they are by other experiments, render the conclusion certain, that such a power is possessed by some terminal organ in the skin or by some one of its elements without the intervention of the nervous system. The former is the more probable hypothesis, for, if otherwise, we should expect to find diversely coloured pupæ corresponding to the different colours in the immediate surroundings, but I do not regard this argument as convincing; and the investigation of the structural basis, and the nature of the physiological processes which take place, afford a subject for research which promises results as interesting as the work itself will be difficult. The

upper and more trustworthy analysis, although depending on insufficient data, further supports the conclusion that the influence acts upon the skin by showing preponderating effects from the colour to which the larger area of skin has been exposed; and the differences are small, both between the effects wrought in the pupæ and between the two areas of skin affected by opposite stimuli.

E. Another rather more elaborate plan of conducting the conflicting colour experiments occurred to me, in which each larva was to be kept separate from the others during Stage III. I made a number of short cardboard tubes with two perforated discs in each of them, one disc at one end, and the other near the middle,

Fig. 3. ($\times 2$.)



b. Black. *c.* Cardboard. *g.* Gilt. *gl.* Glass *s.* Boss of silk. (The black line indicates the black coating, the white margin the gilt coating, while the lines between represent the cardboard substance of the tube.)

dividing the tube into two compartments. The size and shape are shown in fig. 3, which is drawn twice the actual size in all dimensions. In each tube the colour lining one compartment was gold, while black lined the other. The method of application is also shown in fig. 3, *B*, the upper aperture being slipped over the head of the larva, which was then assisted through the lower aperture by the use of forceps; a little glue had been placed on the upper surface of the upper disc, and this was pressed tightly with a slight screwing motion on to the glass, from which the larva was hanging, and in all cases tightly adhered. The larva nearly always remained quiet in the tube, and did not retract its head into the upper compartment. Fearing lest the larva might stretch its head beyond the lower rim of the tube, the external surface, as far as it could be seen from such a point of view, was always of the same colour as the lower compartment, but I do not think that the larvæ ever stretched so far; in fact, the tubes were of such a length that it would have been very difficult for them to do so. All the dimensions were adopted after careful measurement of larvæ in Stage III. The upper compartment was illuminated through the upper aperture, and the lower by the open end of the tube, and also partially through the

space between the larva and the perforation in the lower disc, but this was largely blocked by the larval bristles. The different sizes of the openings through which the compartments were illuminated corresponded to the fact that the light which came down from the window into the upper compartment was far stronger than that which was reflected up into the lower chamber (the glass sheet with the tubes adhering being placed a short distance above an ordinary plain deal table). In order to apply the tubes, the larvæ were induced to suspend themselves from sheets of glass. A number of strips of glass were cut of various lengths, and equal, but narrow, widths (about 4 centimetres), and these were placed together so as to form a number of separate rectangular frames, the angles being secured with gummed paper, a sheet of glass being placed as a roof over the top of each. In this way a number of glass boxes were obtained, having very low sides (4 centimetres), and very large covers. As soon as the larvæ in the stock quitted the food-plant they were turned into these boxes, and, the low sides offering little impediment, they soon mounted to the roof, and prepared for suspension. Some of the boxes were compartmented by another glass strip passing across the centre. In other cases the same results were obtained by placing a sheet of clear glass over a low cylinder of great diameter. In all cases the larvæ of the succeeding subdivisions and those of the next series also were kept in separate glass boxes, compartments, or cylinders. A certain number of larvæ were left free to suspend themselves among the tubes which surrounded the other larvæ, in order to observe the effect of varying proximity to tubes with gilt or black external surfaces as compared with the results produced upon the larvæ inside the tubes. At the same time the results are not very trustworthy, for there is no note as to the time at which the free larvæ suspended themselves, relatively to that at which the tubes were fixed, although in nearly all cases the latter took place at an earlier date.

In the succeeding subdivisions C indicates the time when the suspended larva was covered with a tube, and r that this took place very shortly after suspension began, l being substituted if suspension had begun some hours previously; P indicates pupation, and r that the change had just taken place. In the results, "black below" and "gold below" refer to the lower compartments of the tubes, the upper compartments being always of the opposite colour. Each of the subdivisions below (α , β , &c.) corresponds to a single glass sheet with all the pupæ suspended from it either free or in tubes.

(α) As soon as the tubes had been made on the morning of September 4, a few larvæ, which had not been suspended the night before, were covered (α , β , &c., in the Table below). At a later date larvæ were covered very soon after the beginning of suspension (1, 2, &c. below). The experiment was conducted as follows :—

| Dates, &c | a | b | c | d | e | 1 | 2 | 3 | 4 | 5 | 6 |
|-----------------------|--|----|--|----|----|------------------------------------|-------|-----------------------|------------------------------------|---------------------------------|------------------------------------|
| Sept. 4, 11.30 A.M. . | O1 | O1 | .. | .. | .. | .. | . | . | . | . | .. |
| " 11.45 A.M. . | .. | . | O1 | O1 | O1 | Or | .. | .. | .. | .. | .. |
| " 1.25 P.M. . | .. | . | . | .. | . | .. | Or | .. | .. | .. | .. |
| " 2.48 P.M. . | .. | .. | .. | .. | .. | .. | Or | Or | .. | .. | .. |
| " 4.5 P.M. . | .. | .. | .. | .. | .. | .. | . | . | Or | Or | .. |
| " 4.48 P.M. . | .. | .. | .. | .. | .. | .. | .. | . | Or | Or | Or |
| " 6.5 P.M. . | .. | .. | .. | .. | .. | .. | .. | . | . | . | Or |
| " 12 MIDNIGHT | 1 P r (about 12 hours in tube) | | 1 P r (about 12 hours in tube). | | | .. | .. | .. | About 19 hours in Stage III. | About 19 hours in Stage III. | About 18 hours in Stage III |
| Sept. 5, 9.35 A.M. . | 1 P (more than 12 hours in tube). | | 2 P (more than 12 hours in tube) | | | P | .. | P | P | P | P |
| " 2.30 P.M. . | | | | | | .. | .. | .. | P | P | P |
| Results, Sept. 7 . . | All known about the beginning of suspension is that it had not begun 11.15 P.M., Sept. 3. | | | | | Gold below. | Dead. | Black below. | Gold below. | Gold below | Black below. |
| | 1 (black below) (3), rather golden; 1 dead. | | 2 with black below, both light (3), 1 with gold below, light (3). Little gold on all 3, rather more on the last. | | | (4); gold little for (4). | | Very light (3). | (4); gold little for (4). | Very light (3). | (4); gold little for (4). |

Four more larvæ were left free to suspend themselves on the glass roof, in a strong east light, among the gilded and black tubes surrounding the larvæ tabulated above.

Results :—

1 pupa suspended near 2 tubes, gilt outside very light (3) and little gold.
 1 " " 2 " 1 black and 1 gilt, but nearer the latter light (3) and little gold.
 1 " " 1 tube, black outside light (3) " " "
 1 isolated and fallen down from roof. very light (3) and little gold.

4

Comparing the dates of covering and pupation of the larvæ, *a*, *b*, &c., it is seen that they were covered for a considerable time, but at any rate a portion (and probably all in two cases) of the important earliest part of the stage must have elapsed before they were applied. There is no evidence for any difference between the colours of the pupæ in these tubes corresponding to the different colours of the upper (or lower) chambers, but the pupæ are not so light as those numbered 1, 2, &c., which were exposed to gold in one compartment or the other for the whole of Stage III. In these latter pupæ the three in tubes with the gold chambers below were, on the whole, *slightly* lighter than the others, but there is hardly any difference. The free larvæ produced pupæ which were intermediate in colour between those numbered *a*, *b*, &c., and those numbered 1, 2, &c., and the individual pupæ show traces of slight colour differences which correspond to their respective environments.

The comparison of these results with all others in which the compartmented tubes were used will be shown by means of a tabulated analysis at the end of the last experiment of the kind in the next series.

(*B*) The next experiment was conducted as follows, and consists of three sets of larvæ similar to those described in (*a*) subdivision :—

| Date, &c. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 6 other larvae in compartmented tubes. | 7 larvae free in glass box. |
|---------------------|--|---|-------------------------------|--|--|-------------------------------|--|--|------|---|-----------------------------------|-------------------------------------|--------------------|--|--|
| Sept. 4, 12.30 P.M. | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | The larvae were found suspended, and were covered between 12.5 and 12.30 P.M., Sept. 4. All that is known about the beginning of suspension is that it had not begun 11.15 P.M., Sept. 8. | Left free to suspend themselves among the black and gilt tubes surrounding other larvae. |
| " 12.30 P.M. | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | 12, midnight, Sept. 4 2 had pupated recently, being still green. | |
| " 1.30 P.M. | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | 9.35 A.M., Sept. 5 All the rest had pupated some time. | |
| " 2.30 P.M. | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | | |
| " 4.12 P.M. | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | | |
| " 5.12 P.M. | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | | |
| " 6.12 P.M. | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | | |
| " 9.30 P.M. | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | | |
| " 12 midnight | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | Or | | |
| Sept. 5, 9.35 A.M. | P | P | P | P | P | P | P | P | P | P | P | P | P | | |
| " 2.30 P.M. | P | P | P | P | P | P | P | P | P | P | P | P | P | | |
| Results, Sept. 7. | Black below Very light (8) N.B.—Condition of experiment not rightly carried out; see above | Gold below Light (8) Very little gold | Black below Very light (8) | Gold below Very light (8) Pupa fell down | Gold below (4) Little gold Pupa fell down | Black below Very light (8) | Dead Very light (8) Very little gold | Gold below Very light (8) Very little gold | Dead | Gold below (4) Very little gold for (4) | Black below (4) Little gold | Gold below (4) Pupa fell down | Black below (8) | 4 tubes had the gold compartment below, and contained— 2 . . . (4) 1 very light (3) 1 . . . (3) 2 tubes had the black compartment below, containing— 2 . . . (8) and rather darker than the (8) above One of the (4) alone out of the 6 possessed very considerable gold; very little on all the others | 1 pupa close to tube gilt outside, and much further from 2 black ones, was (4). 8 pupae in a square space, with 8 gilt tubes and 1 black one at its corners, were— 1 . . . (4) 2 very light (3) 1 pupa, near 2 black tubes and further off 2 gold tubes, was light (3) 1 pupa, close to 2 gold tubes, was very light (3) 1 isolated, but nearer gold tubes than anything else, was (8), the darkest of all these free pupae. None of the (4) possessed the normal amount of gold. |

N.B.—There is no note as to presence or absence of special gold in the degrees of colour found in pupae in the tubes with black compartments below; but if this gold had been at all prominent—even normal—it would certainly have been noted.

The results of the above Table are very similar to (α). In the larvæ numbered 1, 2, &c. (omitting 1, because the experiment failed in this case), the pupæ in the tubes with gilt chambers below are a little lighter than those with the black ones below; while greater differences in the same direction are shown in the less trustworthy results obtained from the six larvæ which were covered by the tubes for part of Stage III.; and, as in (α), the latter are on the whole darker than those which had been exposed to gilt surfaces for the whole stage, while the free pupæ are about intermediate, and also seem to show some faint correspondence with the colour of the surrounding tubes.

(γ) Another small subdivision is tabulated below, and only consisted of two larvæ placed in tubes during the whole of Stage III., and of six free larvæ:—

| Dates, &c. | 1 | 2 | 6 larvæ free in glass cylinder. |
|-----------------------|-----------------------------|--|---|
| Sept. 4, 12.25 P.M. . | Cr | .. | These were free to suspend themselves among the black and gilt tubes. |
| " 4 P.M. . . | .. | Cr | |
| " 5, 9.35 A.M. . | P | P | |
| Results: Sept. 7 . . | Black below Dark (3) | Gold below (4) Very pink but hardly any gold | 2 pupæ on side of cylinder and close together, and both (3). 1 on roof close to tube, black outside, (3). 1 on roof, about equidistant from gilt and black tube, (3). 1 on roof near to black tube, dark (3). 1 on roof, far off a black tube, light (3). — 6 |

As far as the evidence goes, the tube with the gilt chamber below produced much the greater effect, but the comparison only depends upon two individuals. The free pupæ cannot well be compared with those above, but they are distinctly darker than those in tubes in subdivisions (α) and (β). The pupæ are very uniform, and on the whole do not afford any clear evidence of colour correspondence with the tubes, but at least half of them cannot have been near enough to be influenced at all.

(δ) This subdivision only consisted of the same two sets as those described in (γ). The experiment is tabulated below :—

| Date, &c. | 1 | 2 | 3 | 4 | 5 | 9 larvæ free in glass box |
|----------------------|---|----------------------------------|---|---------------------------------|---|---|
| Sept. 4, 4.15 A.M. . | Cr | Cr | | | | These larvæ were free to suspend themselves among the black and gilt tubes surrounding the other larvæ. |
| " 6 P.M. . . | Stage III., about 17½ hrs. | Stage III., about 17½ hrs. | Cr | Cr | Cr | |
| " 9.15 P.M. . | | | .. | Cr | Cr | |
| Sept. 5, 9.50 A.M. . | Pr | Pr | .. | Stage III., about 16 hrs. | Stage III., about 16 hrs. | |
| " 1.30 P.M. . | .. | .. | P | Pr | Pr | |
| Results: Sept. 7 . | Gold below Injured, but appears to be light (3) | Black below Light (3) | Black below Light (3) pupa fell down | Black below Light (3) | Gold below Injured, but appears to be rather darker than light (3) | 1 pupa, isolated, was (3) 2 little nearer a tube gilt outside than a black one were both dark (3). 2 equidistant from a black and a gilt tube were both (2). 1 nearer black than gilt tube was (3). 1 some distance from a black tube was dark (3). 1 little nearer gilt than black tube was (3). 1 much nearer black than gilt, quite close to the black, was light (3). - 9 |

The results of the experiments with the tubes seem to be completely uniform, while the free pupæ are on the whole decidedly darker; and, as in (γ), the individuals do not exhibit any evidence of correspondence with the colours of surrounding tubes.

This subdivision concludes the experiments with compartmented tubes in this series; they will be analysed at the close of the description of similar experiments in the next series.

F. A few pupæ were found among the food, &c., of the cylinders containing the stock of larvæ, and these are interesting as affording a further criterion of the colours assumed, when there is little stimulus from surrounding surfaces, towards either the light or dark forms.

In one of the cylinders containing the stock four pupæ were found, of which two were lying on the plain deal floor (one light (3), one very light (3)), while two were suspended from the side (one dark (3), one light (3)).

In the other cylinder also four pupæ were found, of which two were suspended from the food-plant (both (3)) and two were lying on the floor of white paper, but darkened with food-plant and fæces (one dark (3), one very light (3)). One larva was placed in a tube consisting only of the upper gilt compartment, and thus the only black surfaces below were the lower face of the lower disc and the outside of the cylinder. It was not known how much of Stage III. was passed in the tube. The pupa was (3).

Thus 146 pupæ were obtained from this series. Some of the results of the above-recorded experiments are deferred until after the description of similar experiments in the next series.

XIII.—Another large company of larvæ was found, also August 31, on the large nettle-bed near South Hincksey, upon which three of the previous series were also found. These were kept in clear glass cylinders, and were made use of in the following experiments :—

A. Some of the larvæ which were found suspended in the cylinders containing the stock were transferred to gilt and black surfaces for the rest of Stage III.

Thus, on September 4, 12 suspended larvæ were taken from the clear glass cylinders, where they were attached to the food-plant and clear glass roof, &c., and were pinned against a gilt surface facing a strong east light, close to the glass of the window (about 5 centimetres distant). The larvæ were taken as soon after the beginning of suspension as possible, but there were considerable differences in this respect, as the Table indicates. Similarly eight larvæ were pinned against a black surface under conditions which were otherwise exactly similar. The experiment was conducted as follows :—

| Dates, &c. | (a) 12 larvæ transferred from stock to gilt. | | | (b) 8 larvæ transferred from stock to black. |
|--------------------------------|--|-------------------------------|--|---|
| Sept. 4, 3 P.M. . . | . . . | . . . | | 8 suspended larvæ pinned on black surface. |
| " 3.15 P.M. . . | . . . | . . . | 12 suspended larvæ pinned on gilt surface | No change. |
| " 9 P.M. . . | . . . | . . . | No change | " " |
| " 9.15 P.M. . . | 1 just pupated 6 hrs. on gilt | . . . | No change in others . . | " " |
| " 12 MIDNIGHT . | . . . | 1 just pupated 9 hrs. on gilt | " " . . | 1 pupated (less than 9 hrs. on surface) and 1 pupating (9 hrs. on surface). |
| Sept. 5, 9.55 A.M. . . | . . . | . . . | All pupated some hours . | All pupated some hours. |
| Results of comparison, Sept. 7 | (3) . . | Light (3) | Of the 10 pupæ— 2 were (2) 3 " (3) 3 " light (3) 1 was very light (3), unusual gold. 1 was very light (4) with normal gold, but rather dark in other respects, red-dish posteriorly. — 10 | Of the 8 pupæ— 1 was dark (3) 2 were (3) 5 were light (3) — 8 With the normal moderate amount of gold on half the pupæ. |

It is quite certain that the larvæ of which the time of pupation is known only passed the latter half, or even less of Stage III., on the gilt or black surfaces, and it is equally certain that few of the others had been transferred for the whole stage; for, if we assumed that pupation took place in the majority of cases at 5 A.M., September 5, this would only leave 14 hours for Stage III.

It is probably on this account that the colours show so little correspondence with the surfaces on which pupation took place, for there are even two (2) on the gilt surface, although these are compensated by the one (4) and the one very light (3).

B. In the next experiment the compartmented tubes were made use of which were described in Division E. of the last series. These experiments are also divided into subdivisions, each of which corresponds to a sheet of glass with all its suspended pupæ covered and free, and therefore subjected to uniform conditions of illumination.

(α) In the first experiment the larvæ were covered with the tubes for nearly the whole of Stage III., and there were no free larvæ among the tubes. The experiment is shown below :—

| | 1 | 2 | 3 | 4 | 5 |
|----------------------|---|---------------------------------|---------------------------------|--|------|
| Sept. 4, 4.40 P.M. . | All covered with tubes soon, but not immediately, after suspension. | | | | |
| Sept. 5, 9 50 A.M. . | P | P Mostly fallen down | P | P | |
| Results, Sept. 7 . . | Gold below (4) | Gold below Very light (3) | Black below Very dark (3) | Gold below (4) Rather dull, but fair amount of gold | Dead |

Hence the tubes with the gilt chamber below produced much lighter results than those with the gilt chamber above; and it appears to be probable that the larvæ were decidedly influenced towards the light side of normal, and therefore that but little of Stage III. had elapsed before the tubes were applied.

(β) The next experiment included larvæ in tubes for all Stage III., others for part of the stage, and others free among the tubes.

| Date, &c. | 1 | 2 | 3 | 4 | 5 | 4 other larvæ in compartmented tubes. | 5 larvæ free in glass box. |
|-------------------------|------|------|-------------------------------------|---------------------------------------|--------------------------------------|---|--|
| Sept. 4, 6.30-6.45 P.M. | Cr | | | | | Few notes taken, because it was not known how long the larvæ had been suspended when covered | These were free to suspend themselves among the gilt and black tubes surrounding the other larvæ. |
| „ 9.10 P.M. . . | .. | Cr | Cr | | | | |
| „ 11.45 P.M. . . | .. | .. | .. | Cr | Cr | | |
| Sept. 5, 12.40 P.M. . | . | .. | Stage III., 19 hrs. 20 mins. | Stage III., more than 16½ hours | Stage III., 16½ hours | Sept 4, 11.45 P.M., 2 pupated recently Sept. 5, 9.50 A.M., all pupated | Sept. 5, 9.50 A.M., all pupated. |
| „ 2 P.M. . . . | .. | . | | | | | |
| „ 4.30 P.M. . . | .. | .. | Pr | | Pr | | |
| „ 10.55 P.M. . . | P | .. | .. | P | | | |
| Results, Sept. 7 . . | Dead | Dead | Gold below Very light (3) | Gold below Very light (3) | Black below Very light (3) | 2 gold below contained: 1 (4), very light pink and little gold 1 very light (3) 2 black below contained: 1 (2) 1 only partially changed, but apparently a dark (3) | 1 close to 1 tube, gilt outside, was dark (3). 1 good deal further off was (2). 3 quite close to 1 tube, black outside, were: 1 dark (3) 1 . . (3) 1 light (3). These all on glass roof. - 5 |

The results produced after the larvæ had been covered for the whole of Stage III. (i.e. 1, 2, &c.) are all the same, the position of the gilt chamber making no difference, while in those covered for a shorter time the difference is very great, the two pupæ in the tubes with the gilt chamber above being very dark, and supplying the only instance of a (2) in the whole of the experiments with compartmented tubes. It is probable, in fact nearly certain, that the darkness of these two pupæ is at any rate partially due to individual tendencies or to influences which acted before the larvæ were covered. There are no evidences of any colour-relation between the free pupæ and the neighbouring tubes.

(γ) In this, the last experiment with compartmented tubes, I removed the upper

perforated disc from each tube, believing that I had in the other experiments allowed far too much for the greater illumination of the upper chamber. The results of the experiment are shown below :—

| Dates, &c. | 1 | 2 | 3 | 4 | 5 | 7 larvæ free in glass box. |
|------------------------|---|--|------------------------------|---------------------|------|--|
| Sept. 5, 12.5 P.M. . . | Cr | | | | | These were free to suspend themselves among the gilt and black tubes surrounding the other larvæ. Time of pupation unknown. |
| " 2 15 P.M. . . | .. | Cr | | | | |
| " 4 30 P.M. . . | . | .. | Cr | Cr | Cr | |
| " 11 P.M. | | | | | | |
| Results, Sept. 7. | Gold below (4) Very light and pink, but little gold | Black below (5) Rather dull for this stage | Time of pupation Dead | unknown Dead | Dead | 4 on glass roof of cylinder :— 2 isolated, both (4), very light and pink, but little gold. 1 close to a tube, gilt outside, (4) as above. 1 close to 2 tubes, black outside, very light (3); resembling the others, only dotted over with dark pigment. 1 on side, isolated, (4) as above. 2 on floor; both (4) as above. — 7 |

Most unfortunately only two larvæ underwent the final change, and at this time the stock of larvæ was exhausted, and no more could be obtained until another season. Had I been able to find them, I much wished to make a large number of experiments with tubes in which the upper disc was removed. However, as far as the experiment goes, it strongly supports my impression that the predominance of colour-effect produced by tubes with the gilt chamber below is entirely due to the much greater illumination from the wider lower opening. This will be alluded to below in discussing the analysis of all results obtained after the use of compartmented tubes. In this experiment the pupa in the tube with the gilt chamber above was a somewhat dull (5), but nevertheless the only (5) obtained in any of the experiments with the tubes. Among the free larvæ there is some colour-correspondence with the neighbouring tubes, which certainly suggests some influence on the part of the latter. It is interesting to note the extremely light results of experiment (γ) as a whole.

Before concluding the experiments of Series XIII., it is best—now that all the results obtained by the use of tubes have been described—to analyse the colours obtained in all subdivisions in which this method has been employed in this and the preceding series. The analysis is given below :—

SERIES XII., E.— α , β , γ , δ .

| | Degrees of colour. | (1) (3) | Very dark (3) | Dark (3) | (3) | Light (3) | Very light (3) | (4) | (5) | Totals. | |
|------------|--|---------|---------------|----------|-----|-----------|----------------|-----|-----|---------|---|
| α . | Larvae in tubes, } Gold chamber below, rather more skin in black Stage III. } Black " " gold. | .. | .. | .. | .. | .. | 1 | 2 | .. | 3 | { Almost equal, but some preponderating effect follows when the gold chambers are below. Results equal as far as evidence goes; darker than the above. Results intermediate between the two sets compared above. |
| | Larvae in tubes, } Gold " " black | .. | .. | .. | .. | .. | 1 | 1 | .. | 2 | |
| | part of Stage III. } Black " " gold. | .. | .. | .. | .. | .. | .. | .. | .. | .. | |
| | Larvae free among tubes | .. | .. | .. | .. | .. | 2 | .. | .. | 4 | |
| β . | Larvae in tubes, } Gold chamber below, rather more skin in black Stage III. } Black " " gold. | .. | .. | .. | .. | 1 | 2 | 8 | .. | 6 | { Rather preponderating effects when the gold chamber below Decidedly preponderating effects when the gold chamber below; a little darker than the above. Intermediate between the above sets |
| | Larvae in tubes, } Gold " " black | .. | .. | .. | .. | .. | 1 | 1 | .. | 4 | |
| | part of Stage III. } Black " " gold. | .. | .. | .. | .. | 2 | .. | 2 | .. | 4 | |
| | Larvae free in glass box, &c., among tubes | .. | .. | .. | .. | 1 | 3 | 2 | .. | 7 | |
| γ . | Larvae in tubes, } Gold chamber below, rather more skin in black Stage III. } Black " " gold. | .. | .. | 1 | .. | .. | .. | 1 | .. | 1 | { Results very different; great preponderance when the gilt chamber is below Probably normal for free pupae, and the same as the second set. |
| | Larvae in tubes, } Gold " " black | .. | .. | 1 | .. | .. | .. | .. | .. | 1 | |
| | part of Stage III. } Black " " gold. | .. | .. | 1 | 4 | 1 | .. | .. | .. | 6 | |
| | Larvae free among tubes | .. | .. | .. | .. | .. | .. | .. | .. | .. | |
| δ . | Larvae in tubes, } Gold chamber below, rather more skin in black Stage III. } Black " " gold. | .. | .. | .. | 2 | 2 | .. | .. | .. | 2 | { Results equal. Probably normal for free pupae, and darker than the above sets. |
| | Larvae in tubes, } Gold " " black | .. | .. | .. | .. | 3 | .. | .. | .. | 3 | |
| | part of Stage III. } Black " " gold. | .. | .. | .. | .. | 1 | .. | .. | .. | 9 | |
| | Larvae free among tubes | .. | .. | 4 | .. | 5 | .. | .. | .. | 58 | |
| Totals. | Larvae in tubes, } Gold chamber below, rather more skin in black Stage III. } Black " " gold. | .. | .. | 1 | .. | 3 | 3 | 6 | .. | 12 | { Decidedly lighter than in the set next below Lighter than normal. Decidedly lighter than in the set next below; not so light as first set. About normal. Normal, about; darker than all the above sets except the last. |
| | Larvae in tubes, } Gold " " black | .. | .. | 1 | .. | 3 | 3 | 2 | .. | 10 | |
| | part of Stage III. } Black " " gold. | .. | .. | .. | .. | 2 | 1 | 2 | .. | 6 | |
| | Larvae free among tubes | .. | .. | 4 | .. | 5 | .. | 2 | .. | 26 | |
| | | .. | .. | 2 | .. | 5 | .. | .. | .. | 58 | |

SERIES XIII., B.— α , β , γ .

| | Degrees of colour | (1) (3) | Very dark (3) | Dark (3) | Light (3) | Very light (3) | (4) (5) | Totals. | |
|----------|---|---------|---------------|----------|-----------|----------------|---------|--------------|--|
| α | Larvæ in tubes, } Gold chamber below, rather more akin in black nearly all Stage } Black " " " | | .. 1 | | | 1 .. | 2 .. | = 3 = 1 | { Results very different, and the dark pupa in tube with gilt chamber above may be abnormally dark; the others normal. Results equal. Results much stronger when gilt chamber below, but the others are probably abnormally dark for those in tubes. Probably normal for free pupæ, and darker than the above sets, except for the two last pupæ. Stronger results when the gilt chamber was above. Very light pupæ, much the same as the upper of the two sets |
| | Larvæ in tubes, } Gold " " " | | | | | 2 1 | | = 2 = 1 | |
| | Larvæ in tubes, } Black " " " | | | | | 1 1 | 1 .. | = 2 = 2 | |
| | Larvæ in tubes, } Gold " " " | .. 1 | | .. 1 | | | | = 2 = 5 | |
| β | Larvæ free in glass box, &c., among tubes | .. 1 | | 2 1 | 1 1 | | | | { Stronger results when the gilt chamber was above. Very light pupæ, much the same as the upper of the two sets |
| | Larvæ in new } Gold chamber below, rather more akin in black kind of tube, } Black " " " | | | | | | 1 .. | = 1 = 1 | |
| | Larvæ free among tubes | | | | | 1 .. | 6 .. | = 7 = 25 | |
| | Larvæ in tubes, } Gold chamber below, rather more akin in black Stage III. } Black " " " | | .. 1 | | | 3 1 | 8 .. | = 6 = 3 | |
| γ | Larvæ in tubes, } Gold " " " | | | | | 1 1 | 1 .. | = 2 = 2 | { Lighter than the set next below Much lighter than normal Much lighter than the set next below; same as first set Darker than normal Normal except in the number of (4), and darker than all the above sets except the last. |
| | Larvæ in tubes, } Black " " " | .. 1 | | 1 2 | 1 1 | | 6 .. | = 12 = 25 | |
| | Larvæ free among tubes | | | | | | | | |
| | Larvæ in tubes, } Gold chamber below, rather more akin in black Stage III. } Black " " " | | .. 1 | .. 1 | 3 3 | 6 4 | 9 .. | = 18 = 18 | |
| Totals | Larvæ in tubes, } Gold " " " | | .. 1 | .. 1 | 3 3 | 4 2 | 2 1 | = 7 = 7 | { Decidedly lighter than below; the lightest of all. Much lighter than normal. Much lighter than the set below, about the same as the one immediately above Darker than normal. Normal except perhaps in the number of (4); darker than all the above sets, except the last. |
| | Larvæ in tubes, } Black " " " | | | | 1 1 | 2 2 | | = 7 = 38 | |
| | Larvæ free among tubes | .. 1 | | 1 6 | 2 6 | | 8 .. | = 38 = 83 | |
| | Larvæ free among tubes | .. 3 | | | | | | | |

In the above Table it is seen that the difference in the position of the gilt chamber corresponds with a larger difference in pupal colour when the larvæ were covered for part of Stage III. than when they were covered for the whole of it. And the difference is made by the increased darkness of the pupæ of the former set in tubes with the gilt chamber above (especially in XIII.), while the pupæ in the other tubes of the same set are as nearly as possible identical with those in similar tubes, but which had been covered for the whole stage. This is probably due to the fact that the gilt chamber when below was strongly illuminated, and could produce effects even when working for something short of the whole stage—although it is very likely that large numbers of experiments would show that such effects are not so great as when the influence worked for the whole stage, while the less illuminated upper chamber failed to have any effect except when working for the whole stage.

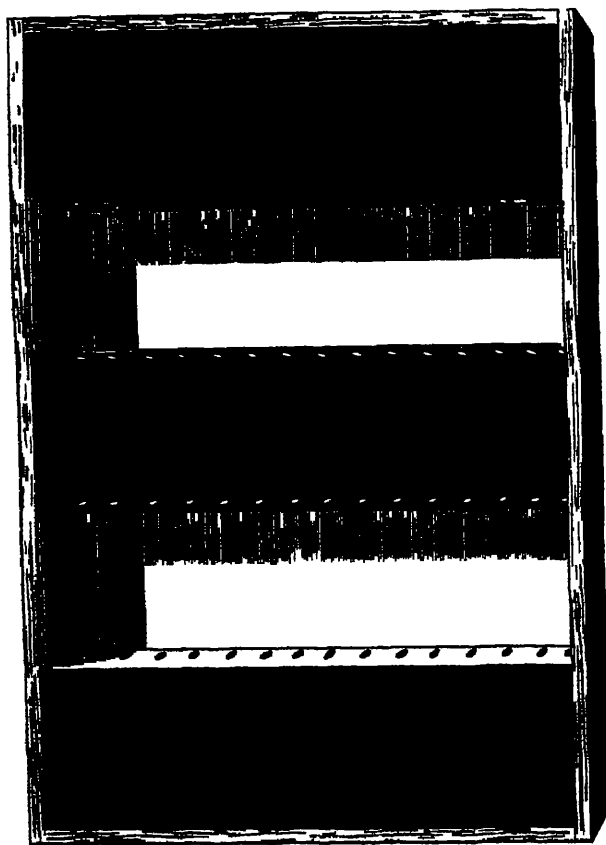
If the results obtained were merely those of the last three lines of the complete total, it would make the conclusion as certain as the conditions of this experiment and the numbers employed could make it—that the influence *does* make itself felt through some anteriorly placed sense-organ situated in the lower chamber, and affected by its colour alone. Accepting the free larvæ as on the whole normal, it is seen that when the head was in black surroundings the pupæ are in no instances as light as the two lightest degrees attained among the normal ones ; but when, on the other hand, the head was in gilt surroundings the pupæ never reach the two darkest degrees of the normal pupæ. But such conclusions are quite upset by the further comparison with the far more trustworthy results of the pupæ which were covered for the whole of Stage III. Here also the pupæ in tubes with the gilt chamber below are rather lighter, but the others, although not equally light, are lighter than the free pupæ. It has been suggested above that the free pupæ are about normal, and I think that this suggestion is confirmed by a comparison with the pupæ found on the food-plant and in the cylinders in which the stock was kept (see below, D.) The influence of a colour—black or gold—felt by a larva must be immensely different according as the latter is inside a tube or outside it, and it may well be that the effects in the last case are so slight as to be often inappreciable when the stimulus has been applied during Stage III. only. Certainly in the above-described experiments the facts of one set of free pupæ seeming to indicate a slight influence are compensated by those of the next, in which the results are highly irregular. I believe, however, that the effects were real in a few instances, although very slight, but there is insufficient evidence for the belief in these experiments alone.

Assuming, then, that the free pupæ are not far from normal, we see that the pupæ in tubes for the whole of Stage III. are lighter, whatever be the position of the gilt chamber ; the gold has a more powerful influence than the black with either system of relative position. But such results are quite inconsistent with the theory that the larval ocelli are influenced by the colour, for it is seen that the gold produces effects when it is shut off from the anterior part of the body together with the head. The

alternative hypothesis—that something in the skin is sensitive to colour influences—seems at first sight to be opposed by the stronger effects which followed the application of the stimulus to the smaller, anterior, skin area. But, in the first place, the difference between the sizes of the two areas was very small, for the anterior part is much swollen before pupation; and, furthermore, I now feel sure that I over-compensated for the greater illumination of the upper chamber. I did not allow for the fact that the larva always spins a film of silk over the glass for a considerable distance round the boss from which it is suspended, and that the transparency is much impaired in this way. Finally, it has been shown that when in the last experiment (XIII., B., γ) there was an equally large opening to the upper as to the lower chamber the gilt surface when above did actually produce greater results than when it was below, although the conclusion is only supported by a pair of pupæ (the sole survivors in this experiment). Although there are so few, the fact that the only (5) out of 83 pupæ was thus produced is certainly important testimony. Finally, when we compare these results with those of the more perfect conflicting colour experiments conducted with the frames previously described (XII., D.), and which will be further mentioned below, we see that the above explanation of the effects produced in the compartmented tubes is probably correct; that such results show that the sensitive surface is not represented by a sense-organ in the head, or with an anterior position only; while, on the other hand, when all the conditions of experiment are considered, the results harmonise well with the converse theory of a general susceptibility of the larval skin to the influence of certain colours.

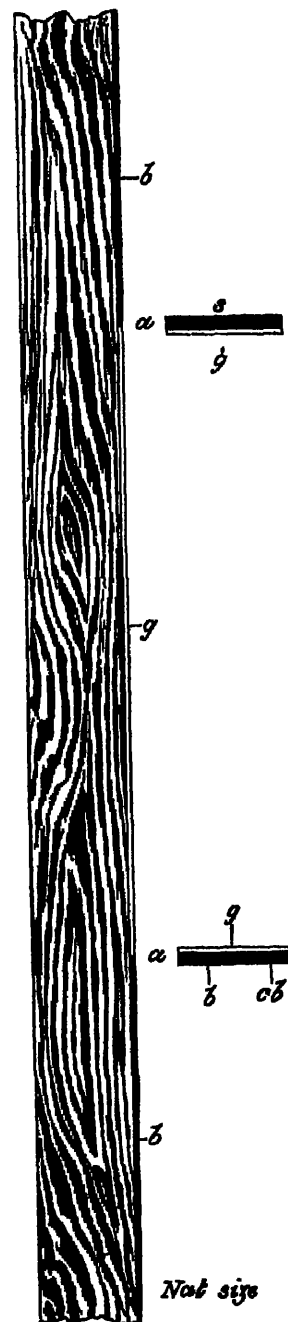
C. Notwithstanding the order in which the above experiments are arranged, the use of compartmented tubes was an earlier contrivance than the frames mentioned in XII., D., and by the end of the last experiments I realised that the latter method was far better, because of the equality of illumination, whether the gilt area was above or below. I therefore made a larger and better frame, of which a drawing is shown in fig. 4 ($\frac{1}{4}$ the real size), while a section is shown in fig. 5 (of the actual size). Upon the frame were pinned all the larvæ which were found suspended in the stock cylinders, for those covered by the tubes were previously placed during Stages I. and II. in special boxes or cylinders. Unfortunately, the larvæ on the frames began to pupate almost at once, and out of the 56 larvæ only seven can have been subjected to the colours for nearly the whole of Stage III., while eight others had been on the frame for about 12 hours (two-thirds of the stage). The experiment is shown below in a tabular form:—

Fig 4.



$\frac{1}{2}$ real size

Fig. 5



| Larva. | A. Head and smaller anterior part of larva in gilt surroundings; larger posterior part in black. | | | | | | | | | | | | | |
|-----------------------------------|--|------------------------------------|-----|-----------|----------|-----------|-----|-----|----------------------------------|---------------|----------|-----------|-----|----------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| Sept. 5, 10.10 A.M. to 11.50 A.M. | .. | .. P | .. | .. P | . | P | .. | .. | . | .. | . | .. | .. | .. |
| " " 11.50 A.M. to 2 P.M. | P | .. | .. | .. | .. | .. | P | .. | P | .. | P | .. | P | .. |
| " " 4.45 P.M. | .. | .. | P | .. | P | .. | .. | .. | .. | .. | .. | Pr | .. | Pr |
| " " 11 P.M. | .. | .. | .. | .. | .. | .. | .. | .. | .. | P | .. | .. | .. | .. |
| Sept. 6, MORNING | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Comparison, Sept. 7 | Injured, light (3) | Very light (3), good deal of gold. | (3) | Light (3) | Dark (3) | Light (3) | (2) | (2) | (3) Rather more gold than usual. | Very dark (3) | Dark (3) | Light (3) | (2) | Dark (3) |

| Larva. | B. Larva in gilt surroundings | | | | | | | | | | | | | |
|-----------------------------------|-------------------------------|------|-----|-------------------------|----------|---|-----|----------|-------------------|---------------|---------------|-----------|---------------|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| Sept. 5, 10.10 A.M. to 11.50 A.M. | P | .. | .. | P | . | P | .. | .. | .. | .. | .. | .. | .. | .. |
| " " 11.50 A.M. to 2 P.M. | .. | .. P | .. | .. | P | .. | .. | .. | .. | .. | .. | .. | .. | P |
| " " 4.45 P.M. | .. | .. | P | .. | .. | .. | .. | .. | P | .. | P | Pr | .. | .. |
| " " 11 P.M. | .. | .. | .. | .. | .. | .. | .. | .. | .. | P | .. | .. | .. | .. |
| Sept. 6, MORNING | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Comparison, Sept. 7 | (4) Normal gold | (2) | (3) | Light (3), unusual gold | Dark (3) | (4) Very brilliant gold, though little of it. | (3) | Dark (3) | Dead, discoloured | Very dark (3) | Very dark (3) | Light (3) | Very dark (3) | (4) Very brilliant gold, though little of it. |

| Larva. | C. Head and smaller anterior part of larva in black surroundings; larger posterior part in gilt. | | | | | | | | | | | | | |
|-----------------------------------|--|-------------|-----|-----------------------------|--|--------------|-----|-----|---|---|-----|-------------------|--|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| Sept. 5, 10.10 A.M. to 11.50 A.M. | .. | .. | .. | .. | P | .. | P | .. | .. | .. | .. | .. | .. | .. |
| " 11.50 A.M. to 2 P.M. | P | P | .. | P | .. | .. | .. | .. | .. | .. | .. | .. | .. | P |
| " 4.45 P.M. | .. | .. | .. | .. | .. | Pr | .. | P | Pr | .. | .. | P | .. | .. |
| " 11 P.M. | .. | .. | Pr | .. | .. | .. | .. | .. | .. | P | .. | .. | .. | .. |
| Sept. 6, MORNING | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Comparison, Sept. 7 | Very dark (3) | Dark (3) | (3) | Injured, probably (3) | (4) Dull, but with good deal of gold | Light (3) | (3) | (2) | (4) Very light pink gold also present | (5) Not much gold for this degree | (3) | Very light (3) | In- perfectly pupated; apparently light (3) | (2) |

| Larva. | D Larva in black surroundings. | | | | | | | | | | | | | |
|-----------------------------------|--------------------------------|-----------------------|-----|-------------|--------------|--------------------------------------|-----|-----|-----------|--|------------------|------|-----|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| Sept. 5, 10.10 A.M. to 11.50 A.M. | P | .. | .. | .. | .. | P | P | .. | .. | .. | .. | .. | .. | .. |
| " 11.50 A.M. to 2 P.M. | .. | P | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| " 4.45 P.M. | .. | .. | P | .. | Pr | .. | .. | .. | .. | .. | P | P | .. | .. |
| " 11 P.M. | .. | .. | .. | Pr | .. | .. | .. | .. | P | .. | .. | .. | .. | P |
| Sept. 6, MORNING | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Comparison, Sept. 7 | (3) | Injured, light (3) | (2) | Dark (3) | Light (3) | (4) Though with little gold | (3) | (2) | Light (3) | Light (3); unusual gold, but very dark pigment | Very dark (3) | Lost | (3) | Lost |

The larvæ were pinned on the frame between 10.10 A.M. and 11.50 A.M., September 5, and several are seen to have pupated almost immediately, and before the process of pinning could be completed.

The pupæ were arranged in four rows, A, B, C, D, and the larvæ were taken from the roof, sides, and food-plant of both cylinders containing the stock of this series. When any group of suspended larvæ was found on any part of the cylinders they were always pinned, one after the other, on all the rows, viz., first A 1, then B 1, C 1, D 1, and then beginning again at A 2, and so on. In this way any tendencies possessed by the individuals of each group (due to time of suspension, reciprocal effects of the black larval surfaces, &c.) were, as far as possible, compensated, and we can trace the effects of this method in the results. Thus No. 8 in each row is remarkable for exceptional darkness (three (2) and one dark (3)), and each of the four larvæ was found to have pupated at the same time of examination (11 P.M., September 5).

The chief results of this Table are analysed below :—

| Degrees of colour :— (1) Darkest and least golden (5) Lightest and most golden. | (1) | (2) | Very dark (3) | Dark (3) | (3) | Light (3) | Very light (3) | (4) | (5) |
|--|-----|-----|---------------------|-------------|-----|--------------|----------------------|-----|-----|
| Analysis of pupæ which had changed recently at 11 P.M., or after that time, and there- fore had been on frame about 12 hrs. or more in all cases | | | | | | | | | |
| A | .. | .. | 1 | 1 | . | 1 | . | .. | .. |
| B | .. | .. | 1 | .. | .. | 1 | .. | .. | .. |
| C | .. | 1 | .. | .. | 1 | 2 | . | 1 | 1 |
| D | .. | .. | .. | 1 | .. | 3 | .. | .. | . |

The comparison between the larvæ exposed to the influence of conflicting colours—viz., A and C—is in favour of the view that the larval skin supplies the sensitive surface which is affected by light of certain colour. The results produced on the larvæ upon the single colours, black or gold, viz., B and D, are less satisfactory, but the numbers are very small. If the eight larvæ which must have been longest on the frame are analysed alone, we obtain the following results :—

| Degrees of colour :— (1) Darkest and least golden. (5) Lightest and most golden. | (1) | (2) | Very dark (3) | Dark (3) | (3) | Light (3) | Very light (3) | (4) | (5) |
|--|-----|-----|---------------------|-------------|-----|--------------|----------------------|-----|-----|
| Analysis of pupæ which had changed on the morning of Sept. 6, and which had therefore probably in most cases passed nearly the whole of Stage III. on the frame | | | | | | | | | |
| A | .. | .. | 1 | .. | .. | .. | .. | .. | .. |
| B | .. | .. | 1 | .. | . | .. | .. | .. | .. |
| C | .. | .. | .. | .. | 1 | . | 1 | .. | 1 |
| D | .. | . | .. | .. | . | 2 | . | .. | .. |

Here, again, the comparison between A and C affords very complete support to the above-mentioned theory as to the larval sense-organs which are affected; but B and D are also unsatisfactory, and it is very likely that one or more out of these three larvæ

in the two rows may have pupated soon after 11 P.M., September 5, and thus may have only spent two-thirds of Stage III. on the frame. I do not urge this because of the confliction of the results with A and C, but because of the earlier transference experiments, in which results were distinctly shown to follow from exposure during the whole of Stage III., and this in spite of the previous influence of colours tending to cause an opposite effect (see results of Series IX., B.). It now remains to add together the most trustworthy results of this experiment as shown in the last Table, and the most trustworthy results of the similar experiment in the last series (XII., D.).

| Degrees of colour | (1) | (2) | Very dark (3) | Dark (3) | (3) | Light (3) | Very light (3) | (4) | (5) | |
|--|-----|-----|------------------|-------------|-----|--------------|-------------------|-----|-----|-----|
| A. Larger skin area in black, smaller in gilt for Stage III | .. | .. | 1 | 1 | 1 | .. | | .. | .. | = 3 |
| B. Entirely exposed to gilt for Stage III. | .. | . | 1 | | . | .. | .. | 5 | 1 | = 7 |
| C. Largest skin area in gilt, smaller in black for Stage III | .. | .. | .. | .. | 1 | 2 | 1 | .. | 1 | = 5 |
| D. Entirely exposed to black for Stage III | .. | .. | .. | .. | . | 5 | 2 | 1 | .. | = 8 |
| | | | | | | | | | | 23 |

The comparison between A and C, and between either of them and B, entirely supports the above-mentioned conclusion, while such results cannot be made to harmonise with the theory of a sense organ of this kind in the head. The results of D remain as a difficulty under any theory, and I can only account for it by supposing that the individuals were unusually light.

At the close of these long series of conflicting colour experiments, entirely directed towards the settlement of the important question of the position of the sensory surface, I must again point out that all the results which were to be gauged have been acquired from the influences working during Stage III. only, and very often for only part of it, and that all previous experiments of the kind have pointed towards the conclusion that such results must be highly irregular, and can only be brought to bear as evidence by the use of large numbers. I could not, and did not, expect that the results would be more regular than they have proved to be, but I expected sufficiently clear results to confirm or to upset the conclusions arrived at from the numerous blinding experiments, and I think that the results have ended in as complete a confirmation as the necessarily limited conditions of experiment could be anticipated to produce. It will be unnecessary to further summarise any of the conflicting colour-experiments.

D. Finally, towards the close of the experiments, 20 pupæ were found on the food-

plant or floor of the cylinders in which the stock was kept. These are useful as affording criteria of the normal tendencies of this series. By "normal" I mean, in such cases, the tendency which manifests itself when the larva is placed among colours to the influence of which it is not sensitive; and the resulting pupæ deserve the name "normal" for another reason—because they are generally the commonest forms met with. But that they are the commonest merely implies the continual selection of certain surroundings which do produce effects on the larva, so that we see that the continual repetition of an influence of a certain kind, always, generation after generation, producing its corresponding effects, may gradually wear out for itself a line of least resistance along which the formation of pupal colours will always tend to travel, not only when the appropriate stimulus is present, but also in the absence of any colour which can act as a stimulus to the larva. Hence, in Mrs. BARBER's paper, we learn that by far the commonest forms of the pupa of *Papilio nireus* are deep green, because they nearly always pupate among green leaves, and thus the deep green pupæ, again and again formed, became the "normal" form, or that variety which most individuals will assume in the absence of anything which can be a stimulus. Accordingly, we find in Mrs. BARBER's experiments that pale green and yellowish and purplish-brown all acted as stimuli and produced pupæ of corresponding colours, but that scarlet could not act as a stimulus (except to the formation of a small part of the colour), and that the resulting pupæ were not scarlet, but were of the commonest deep green colour. It is interesting to note the completeness of the failure of the stimulus in this instance, for the purplish-brown pupæ, if produced, would have been far less conspicuous on the scarlet cloth than the pupæ which were actually produced. And similar facts are to be found in these experiments upon *V. urtica*, when the larvæ were surrounded by green surfaces, which, in this species, do not act as stimuli.

Therefore it is of great interest to collect careful notes of the pupæ suspended from the food-plant, especially when experiments have been made upon others of the same series. The pupæ of this series were of the following colours:—

| | |
|---|--|
| Of the 15 pupæ suspended from the food-plant, 5 were . . (4), mostly with normal gold, but rather dull otherwise. | |
| 6 „ light (3). | |
| 4 „ . . (3). | |
| — | |
| 15 | |

| | |
|--|------------------------|
| Of the 5 pupæ lying on the white paper floor, on which food and dark excreta were also lying | 1 was . . (4), golden. |
| | 1 „ light (3). |
| | 1 „ . . (3). |
| | 1 „ dark (3). |
| | 1 „ . . (2). |
| | — |
| | 5 |

In these results the effects of the white paper floor, although greatly obscured, must not be overlooked as acting upon the pupæ on the food-plant as well as on the floor itself. In this case, of course, the colour exerted its influence during the whole of the preparatory period.

Thus 118 pupæ were obtained from this series, and 598 pupæ altogether from Series III.—XIII., both inclusive, viz., in all the series which correspond respectively to single companies of larvæ. In addition to these, 82 pupæ were obtained from the mixed companies of Series II., and at least 20 in Series I., making a total of 700 pupæ examined, and affording the results upon which the conclusions of this paper are based.

RECAPITULATION AND CONCLUSIONS.

It is now necessary to analyse the results of all the experiments in the above series in which larvæ have been exposed to surroundings of different colours during the whole of the preparatory period, or, at any rate, during Stages II. and III., in order to present the entire proof of the influence of certain colours upon the larvæ as contrasted with the powerlessness of others. Incidentally, the negative results of the blinding experiments will be brought out at the same time. The tabular analysis becomes more complex after the earliest experiments because of the further subdivision of the degree of colour represented by (3); but, at the same time, all the shades of difference included under this one degree are together only equal to any one of the other degrees. The colours will be taken in the order in which they were tested. The effects of crowding, &c., cannot be gone into in the following Tables, although they are necessarily excluded in certain cases.

1. ORANGE :—There was no influence shown in the one experiment in which this colour was used.

2. GREEN.

| Degrees of colour. | (1) | (2) | (3) | (4) | (5) | |
|---|-----|------|------|-----|-----|------|
| Series II. Division C | .. | .. | 5 | 1 | .. | = 6 |
| " " D. | 1 | 3 | 9 | .. | .. | = 13 |
| " " E. | .. | 1 | 3 | .. | 1 | = 5 |
| " " F. (blinded) | .. | 4 | 1 | .. | 1 | = 6 |
| " " H. | 1 | .. | 5 | .. | 1 | = 7 |
| Series III. Division D. | .. | .. | 2 | .. | .. | = 2 |
| Totals | 2 | 8 | 25 | 1 | 3 | = 39 |
| Results expressed as percentages of the total | 5.1 | 20.5 | 64.1 | 2.6 | 7.7 | |

These results show the indifference of the larvæ to the green surroundings very clearly. It is most probable that the slight predominance of the darker forms over the lighter is due to the dim light in the shaded cylinders.

4. BLACK.

| Degrees of colour. | | | (1) | (2) | Dark (3) | (3) | Light (3) | (4) | (5) | |
|--|---|--|------|------|----------|------|-----------|-----|-----|-------|
| Not in black all period | { | Series II. Division G. . . | 1 | .. | .. | .. | .. | . | .. | = 1 |
| | | " III. " C. . . | .. | 2 | .. | 2 | 1 | 1 | .. | = 6 |
| | | " IV. " A. . . | 3 | 4 | 4 | 1 | .. | .. | .. | = 12 |
| | | " IV. " B. . . | 5 | 17 | 13 | 1 | .. | .. | .. | = 36 |
| | | Groups 1 and 2 | | | | | | | | |
| Same condi- tions | { | Series VIII. Division A. Subdivision α . Blinded | .. | .. | . | 1 | 7 | .. | .. | = 8 |
| | | Series VIII. Division A Subdivision β . Normal | .. | .. | 3 | 1 | 4 | .. | . | = 8 |
| | | Series IX. Division B. Group 2 | .. | 1 | .. | 5 | .. | 1 | .. | = 7 |
| Same condi- tions | { | Series XII. Division A. Sub- division α . Black in dark- ness | 2 | 2 | 4 | 3 | 2 | .. | . | = 13 |
| | | Series XII. Division A. Sub- division β . Black in light | .. | 3 | 3 | 8 | .. | .. | . | = 14 |
| Totals | | | 11 | 29 | 27 | 22 | 14 | 2 | .. | = 105 |
| Results expressed as percentages of the total | | | 10.5 | 27.6 | 25.7 | 21.0 | 13.3 | 1.9 | .. | |

These results show the powerful effects of the black surroundings quite as much in the absence of pupæ in column (5), and the presence of only 2 in (4), as in their abundance in the other columns.

5. WHITE.

| Degrees of colour. | | (1) | (2) | Dark (3) | (3) | Light (8) | 4) | (5) | |
|---|--|-----|-----|----------|------|-----------|------|-----|------|
| Same conditions not quite same | Series II. Division J Sub-division α . Unblinded | .. | .. | .. | 1 | .. | .. | 3 | = 4 |
| | Series II. Division J. Sub-division β . Blinded | .. | 2 | | 1 | .. | . | 1 | = 4 |
| | Series III. Division A. Unblinded | .. | . | .. | 5 | 4 | 4 | 2 | = 15 |
| | Series III. Division B. Blinded | . | 3 | .. | .. | 1 | 2 | 2 | = 8 |
| | Series IV. Division B Group 3. (But not in white for the whole period) | .. | .. | 5 | .. | .. | .. | .. | = 5 |
| Same conditions | Series IV. Division C. . . | .. | 1 | 2 | 8 | 4 | 1 | .. | = 16 |
| | Series IV. Division E. Sub-division α . Unblinded | .. | .. | 2 | 1 | 2 | 1 | . | = 6 |
| | Series IV. Division E. Sub-division β . Blinded | .. | .. | 1 | 3 | 1 | .. | .. | = 5 |
| | Series V. Division B. Cylinders 2, 3, 6 | .. | .. | .. | 1 | 2 | .. | .. | = 3 |
| Same conditions | Series V. Division C. Globes with normal larvæ | .. | .. | .. | 3 | 1 | 2 | .. | = 6 |
| | Series V. Division C. Globes with shorn larvæ | .. | .. | .. | 2 | 3 | 1 | .. | = 6 |
| Same conditions | Series V. Division D. Sub-division α . Shorn. | .. | .. | 1 | .. | 1 | 1 | .. | = 3 |
| | Series V. Division D. Sub-division β . Normal | .. | .. | .. | .. | 2 | .. | .. | = 2 |
| Same conditions | Series VI. Division A. Sub-division α . Unblinded | .. | 1 | 3 | 3 | 4 | 1 | .. | = 12 |
| | Series VI. Division A. Sub-division β . Blinded | .. | .. | 2 | 5 | 1 | 3 | 1 | = 12 |
| Same conditions | Series VI. Division B. Globes with unblinded larvæ | .. | .. | 2 | 4 | 4 | 1 | .. | = 11 |
| | Series VI. Division B. Globes with blinded larvæ | .. | .. | 3 | .. | 4 | 4 | .. | = 11 |
| Same conditions | Series IX. Division A. Isolated larvæ | .. | .. | .. | .. | .. | 2 | 2 | = 4 |
| | Series IX. Division A. Crowded larvæ | .. | .. | .. | .. | 10 | 2 | .. | = 12 |
| Totals | | .. | 7 | 21 | 37 | 44 | 25 | 11 | 145 |
| Results expressed as percentages of the total | | 0 | 4.8 | 14.5 | 25.5 | 30.3 | 17.2 | 7.6 | .. |

These figures show the strong effects of white surroundings in producing gilded pupæ.

6. GILT.

| Degrees of colour. | | (1) | (2) | Dark (3) | (3) | Light (?) | (4) | (5) | |
|--|---|-----|-----|----------|------|-----------|------|------|------|
| Same conditions | Series II. Division I Sub-division α . Unblinded | | .. | . | 1* | .. | 1 | 2 | = 4 |
| | Series II. Division I. Sub-division β . Blinded | .. | .. | . | 1 | .. | .. | 1 | = 2 |
| Same conditions in the two subdivisions of each pair | Series IV. Division D. | | 1 | 1 | 5 | 2 | 1 | 1 | = 11 |
| | Series V. Division A. Sub-division α . Shorn | .. | .. | .. | .. | 1 | 2 | 1 | = 4 |
| | Series V. Division A. Sub-division β . Normal | . | | .. | .. | 1 | 3 | .. | = 4 |
| | Series VII. Division A. Subdivision α . Blinded | .. | .. | .. | .. | 1 | 4 | 1 | = 6 |
| | Series VII. Division A. Subdivision β . Normal | .. | .. | .. | | 4 | 1 | .. | = 5 |
| | Series IX. Division B Group IV. | .. | .. | .. | .. | 5 | 7 | 8 | = 20 |
| | Series X. Division A. . . . | .. | . | .. | .. | .. | 5 | . | = 5 |
| | Series XII. Division B. Subdivision α | | . | 1 | .. | 2 | 3 | .. | = 6 |
| Totals | | .. | 1 | 2 | 7 | 16 | 27 | 14 | 67 |
| Results expressed as percentages of the total | | 0 | 15 | 3.0 | 10.4 | 23.9 | 40.3 | 20.9 | . |

These figures show that the gilt surroundings have much more powerful effects than the white surroundings in producing gilded pupæ.

It will now be of interest to place the percentages of these various colours below one another to indicate their differences as strongly as possible.

| Degrees of colour | (1) | (2) | Dark (3) | (3) | Light (3) | (4) | (5) | Numbers of pupæ obtained. |
|-----------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|---------------------------|
| | per cent. | per cent. | per cent. | per cent. | per cent. | per cent. | per cent. | |
| 2. Green surroundings | 5.1 | 20.5 | 64.1 | 64.1 | 64.1 | 2.6 | 7.7 | 39 |
| 3. Black " | 10.5 | 27.6 | 25.7 | 21.0 | 13.3 | 1.9 | 0 | 105 |
| 4. White " | 0 | 4.8 | 14.5 | 25.5 | 30.3 | 17.2 | 7.6 | 145 |
| 5. Gilt " | 0 | 1.5 | 3.0 | 10.4 | 23.9 | 40.3 | 20.9 | 67 |
| Total . . . | .. | .. | .. | .. | .. | . | .. | 356 |

In the above lists the effects of blinding and snipping off the bristles are also recapitulated. The transference experiments and the conflicting colour experiments have been already systematised as far as it is advantageous to do so; for the details are of paramount importance, being absolutely necessary for the interpretation of the

results of experiments in which the colours were only applied for a small part of the time during which the larva is sensitive.

Experiments upon Vanessa atalanta.

Series 1.—Four larvæ of this species were kindly sent to me by Mr. H. L. SURREAGE, and on September 1 two of them were placed in a small glass cylinder surrounded and roofed with two layers of black tissue-paper, and with a black floor. One larva was a dark variety and probably in the stage before the last, while the other was a light variety and in the last stage. At the same time two other similar larvæ—a dark and a light variety, both in the last stage—were placed in one compartment of the gilt box previously described.

Sept. 2. The light-coloured larva in the dark had pupated some few hours. The other larva died.

Sept. 4, 4.20 P.M. The light larva in the gilt box had just suspended itself to the roof.

Sept. 5, 10.38 A.M. The light-coloured larva was pupating. Therefore Stage III. was almost exactly 18 hours in the case of this larva, both limits being obtained with unusual precision. The dark larva pupated subsequently.

Series 2.—Mr. SURREAGE kindly sent me many other larvæ of this species, and of these four nearly mature dark larvæ were (September 10) placed in a small cylinder with a gilt roof and gilt background, extending round half of the internal circumference of the cylinder, the clear side being turned towards a strong light.

On September 14 all had pupated, one upon the food-plant and three upon the roof. Stage III. was estimated at about 20 hours in one case, while in another it must have been at least 24 hours in duration.

At about the same time a few other nearly mature larvæ were placed in a darkened cylinder with black surroundings, and two pupæ were obtained from this experiment.

Three pupæ were obtained from another set of larvæ in a clear glass cylinder roofed with white muslin.

Results.—Thus altogether 6 pupæ were obtained in gilt surroundings, 3 in black, and 3 in clear glass with a white muslin roof. The 9 pupæ of Series 2 were all compared together on September 30, while the 3 pupæ of Series 1 had been compared much earlier and the imagines had emerged. The results, however, appeared to be very uniform.

The pupæ in the gilt surroundings were all very golden for this species, with the magnificent lateral triangular golden patch on the fourth abdominal segment, and with the four dorsal golden patches and sub-dorsal golden tubercles especially bright upon the metathorax and first abdominal segment. The most golden of the

4 pupæ in Series 2 was figured, and is shown in fig. 13, $\times 2$, Plate 26, but there was not much difference between them.

The pupæ in black surroundings were immensely different, the ground-colour being much darker, and the only golden appearance being on the tips of the sub-dorsal tubercle, spreading a very little on to the sides of the latter, while none reached the general surface of the pupa. In Series 2 one pupa was much darker than the other, and in it most of the tubercles have no golden colour on their apices, which are merely of a lighter colour than the rest of the tubercle. This pupa was figured, and is shown in fig. 12, $\times 2$, Plate 26.

The pupæ in the clear glass cylinder were intermediate between the two above-described varieties, but were nearer to the golden ones.

These results are extremely interesting in their specific peculiarities no less than in their harmony with the effects wrought upon the pupæ of *V. urticae*. As far as the experiments went there was nothing at all comparable to the amount of golden colour common upon the varieties of *V. urticae* represented by (4) and (5), although the difference between the two forms, caused by the gilt and black surroundings respectively, was exceedingly marked, and the gilt appearance, though limited in amount, was perhaps more brilliantly metallic than in *V. urticae*. The length of Stage III. seems to be about the same as in the latter species.

The Biological Value of the Gilded Appearance in Pupæ.

It has been long observed by many entomologists that the excessively gilded pupæ which are sometimes found in nature almost invariably contain the parasitic larvæ of the Ichneumonidæ, although Mr. T. W. Wood has proved that this is not necessarily the case. I need hardly say that my own brilliantly golden pupæ, produced by experiment, were entirely healthy, and I had no instance of the presence of parasites in any of them. Large numbers of them were allowed to develop into Butterflies, and these finally emerged in a perfectly normal manner. However, three pupæ of *Vanessa urticae*, found wild upon the food-plant during the past season (1886), were more golden than is usual in nature, and all three contained parasites. The contrast between the golden pupæ produced by gilded surroundings and by disease certainly indicates that the former is the normal association, the latter being probably merely incidental. It is probable that the diseased state of the larva in some way prevents the formation of pigment in the pupa, and then the golden appearance is formed which is always normally associated with the absence of pigment. It is quite clear that these interesting results of abnormality offer us no explanation whatever of the normal use of the gilded appearance. The first suggestion was made by Mr. T. W. Wood in the previously quoted paper—that a gilded pupa does not resemble any object which is of interest to the enemies of its class; but, looking more like “a piece of gold or brass than anything else,” is likely to be passed unnoticed.

The resemblance to brass or gold can hardly be of value, because the only one of these substances which occurs in nature is not sufficiently abundant to offer a model for imitation which is likely to be of any service to the insect; and the same objection holds good against any metal or metallic sulphide, although, as far colour or lustre is concerned, the resemblance to such a substance as iron pyrites would be admirably adapted for protective purposes.

But it is hardly enough to say that the gilded appearance is unlike anything which is usually of interest to insect-eating animals. It is certainly necessary in addition to point to some substance in the surroundings which is also of no interest, and which the pupæ are protected by resembling. And such a substance is, doubtless, found in the glittering mineral mica, which is often metallic and golden in appearance, and which is very widespread and abundant. The shape of the chrysalis of the *Vanessidæ* is very angular, and strongly resembles a mineral surface, and the usual appearance of the scattered golden patches on a grey ground is exactly the effect produced by the manner in which the flakes of mica occur scattered among other less brilliant minerals in granite and other rocks. It has been shown in the experiments that the excessively golden appearance is only produced in normal pupæ when the surroundings are correspondingly brilliant, and such a stimulus would of course be provided by an unusual abundance of mica flakes, or of exceptionally large crystals of this mineral. Furthermore it has been shown that the tint of the lustrous pupæ varies with the colour of the surroundings, being generally silvery when white paper was used, and golden when a gilt surface was employed. Hence the various tints of mica, white and silvery or dark and golden, would produce the corresponding protective shades of colour on the pupæ. When the brilliant lustre of mica or other minerals of recently fractured and exposed rock-surfaces is dimmed by the process of weathering and growth of lichens a grey colour is produced which would act as a stimulus for the darker varieties of pupa, while the darkest would be formed in the deep shadow of irregular cavities and furrows. Hence the pupæ of the *Vanessidæ* have two chief varieties to correspond with the two chief conditions of their mineral surroundings—the brilliant exposed and the grey weathered conditions—while any intermediate result would be formed by any intermixture of the stimuli. In this country we do not see the brilliant metallic pupæ of *V. urticæ*, because in our moist climate the rock-surfaces become grey and weathered almost directly, and because man has offered such facilities for pupation by the erection of walls and houses, which also quickly become grey or are built of some colour (*e.g.*, red brick) which probably does not act as a stimulus. But so perfect is the protection in the natural state that it is extremely rare to find the pupæ of these most abundant insects anywhere except upon walls and houses, which, being plane surfaces, do not conceal the angular forms of the pupæ. But the very shape which renders them conspicuous on these artificial mineral surroundings is eminently protective against almost any natural surface of rock. The susceptibility of the species remains, as

these experiments have shown, and the uniformity of pupal colour seen in England merely follows the uniformity of colour in the surfaces selected for pupation.

It is probable that this protective resemblance to mineral surfaces is very ancient, and it must have been acquired in a dry country, where an exposed rock-surface did not weather for a long time ; and it may even date from a period when many of our modern aggressive vegetal types had not arisen, and when the predominant green colour of the vegetal kingdom contributed less to the total appearance of land-surfaces. And the kind of food-plant may have assisted in causing the protective resemblance to surrounding rocks, for the common ancestor of all those Rhopalocera which have gilded pupæ may have fed on low herbaceous plants, which might have withered in the hottest part of the year, and upon which any pupa which protectively resembled the green plant would be necessarily conspicuous. Certainly, our three commonest *Vanessidæ* pass the pupal stage in the summer, and all feed upon nettle ; but it would probably need a far larger amount of knowledge of the life-histories of larvæ than we possess to make such a comparison as would lead us to conclude as to the exact conditions under which this specialised form of coloration originally arose. It is interesting, and confirmatory of the above explanation of the biological value of this appearance, to consider the way in which certain *Vanessidæ* have adapted themselves to the gradual predominance of green in their surroundings. *Vanessa Io* has a green form which, it has been already shown, is commonly produced when pupation takes place among the leaves of its food-plant, and the amount of gilding upon this variety is not such as would attract attention, for it is not nearly so brilliantly lustrous as that described in *V. urtica* ; and, furthermore, the truly gilded form of the healthy pupa has probably never been obtained, for no one has yet subjected it to gilt surroundings. Hence, when it is said that the green form is more golden than the other variety, it is merely compared with the grey pupa produced by dark mineral surroundings, in which there is hardly any gilding, or none at all. *Vanessa atalanta* has no green form, and when it pupates on the food-plant it commonly attaches itself to the roof of a tent made by the larva, by spinning one or more leaves together. The late Mr. EDWARD NEWMAN describes a most interesting point in this method of concealment which is often (although I believe not always) adopted. He says ('British Butterflies,' 1871, p. 63) : " When full-fed it constructs a somewhat more elaborate retreat ; it gnaws through the petiole of a leaf, or eats the main stalk of the nettle within a few inches of the top, not quite separating it ; the part thus almost separated falls over and completely withers, and this withered portion is formed into a compact retreat ; from the roof of this the caterpillar suspends itself by the anal claspers, and in two days becomes a chrysalis." This is an exceedingly interesting fact : the pupa has no green form, but the larva arranges matters so that the dark pupa shall not be surrounded by living green, but by dark withered leaves, and even when it does not gain this additional colour-relation it is well concealed in the tent which the larva has made. But *V. atalanta* very commonly pupates on mineral surroundings after

having wandered from the food-plant, and under these circumstances it makes no attempt at concealment, but hangs freely suspended against a background with which it harmonises in colour. The contrast between the larval habits when it selects vegetal or mineral surroundings for pupation suggests very strongly that its resemblance to the latter is ancestral, while its somewhat laborious adaptation to the former has been far more recently acquired. *V. urticae*, however, has no green form like *V. Io*, and has not the protective habit of *V. atalanta*, and I find that in Nature it exhibits the strongest disinclination to pupate on the food-plant. In confinement this is also true if it be provided with a surface up which it can readily climb, but the commonest cages are made of glass, which is ascended with difficulty and after foothold has been ensured by spinning a silken surface over the glass. In spite of this, the vast majority of larvæ do ascend the glass sides, and become suspended from the roof. I must also add that the late Mr. EDWARD NEWMAN states of this larva ('British Butterflies,' p. 53) that it prefers for pupation the underside of a nettle-leaf to other situations which he describes as sometimes selected. I can only say of this statement that it is entirely contrary to my experience. It is possible that Mr. NEWMAN's opinion may have been partially or entirely derived from watching the larvæ in confinement. Among the 13 series of larvæ of this species, described above, it will be found that many were only the scanty remnants of companies, and in nearly all these cases I had previously seen the larvæ, and knew that the companies were large. I made it a practice to leave the larvæ on their food-plant as long as possible, in order to save the trouble of rearing them, and to ensure healthy pupæ. In this way, by miscalculating the time at which they would become adult by some hours, I often came in time to find only a few larvæ remaining, all the others having wandered away in search of a surface on which to pupate. Being very anxious for all the material I could get, I always searched the nettle-beds most carefully, and if any larvæ had been suspended, or had pupated on the food-plant, they would almost certainly have been detected. But throughout the whole of the season I only found three pupæ in this position, and no suspended larvæ, although it would be easy to calculate the number of larvæ which had pupated; when I searched their food-plant there must have been very many hundreds. And the history of these three pupæ is important.

(1.) Was found August 22, 1886, on the large nettle-bed near South Hincksey, on which portions of two companies of larvæ were found on the same day. The pupa was a very brilliant (5), but the lustre was silvery rather than golden, and the pupa looked unhealthy. About September 12 numbers of small Ichneumon flies emerged from the pupa case. Mr. E. A. FITCH kindly named the species for me; it was *Pteromalus puparum*, a common parasite of the Vanessidæ.

(2.) August 31, another pupa was found on the same nettle-bed near South Hincksey, but it was empty and perforated by the Ichneumon flies, which had already escaped. It had evidently been very golden and probably like the last.

(3.) The last pupa was found September 16 at Seaview (Isle of Wight) on a nettle-bed from which all the larvæ had disappeared, but upon which an evidently large company had been. The pupa was an exceptionally golden (3), but nevertheless it was dark in the parts which were not golden. The pupa looked unhealthy and did not undergo development; in the winter it had evidently been dead for a long time, and, breaking it in two, I found that it was completely filled with the larvæ of *Ichneumon* flies.

Hence the only exceptions I was able to find are readily explicable: they were all diseased, and doubtless the process of pupation was hurried on by their abnormal condition.

But, while it seems thus probable that the biological value of the gilded appearance was primarily due to its resemblance to glittering mineral surroundings, it is almost certain that in the course of time it has come to be used for other purposes. The most obvious of these is that it may act as a "warning" colour, indicating the possession of unpleasant qualities (taste or smell). But that this cannot be the original use is, I think, shown by the following considerations:—(1) The extreme specialisation of the means by which the colour is produced, and the fact that it is probably less effective than the crude combinations and startling contrasts of pigment colours upon which other warning colours depend; furthermore, belonging to such a very different type of colour, it does not follow the principle of a general resemblance between the various warning colours, which offers to insect-eating vertebrates as short and easy an educational career as possible. (2) The fact of the colour-relation itself, in which these gilded appearances have been shown to play a very important part. Now an adjustable colour-relation is the very highest and most complete means by which protective resemblance to surroundings can be produced, taking cognisance, as it does, of the inevitable differences between the surroundings of different individuals. But the object of a warning colour is to render its possessor as unlike its surroundings as possible, and hence it is something very essentially distinct from a colour which causes it to resemble its surroundings in the most perfect of all ways.*

Nevertheless there is nothing to prevent the one from changing into the other in the course of time. And the present condition of any animal is such a compound phenomenon, made up of so many modified and unmodified habits and structures connected with other older modes of life, interwoven with those which are especially related to its present needs, that it would not be surprising to find that a pupa which made use of the gilded appearance as a warning, in order to render it conspicuous, nevertheless retained something of the ancestral significance of the appearance in responding to some unusual stimulus caused by gilded surroundings. This suggestion is supported by the case of *Acræa esebria*, alluded to below.

* Since the above was written I have tested the golden pupæ of *V. urtica* by offering them to insect-eating animals. There is, evidently, nothing distasteful about them, for the most scrupulous of all insect-eaters yet tested—a Marmoset—ate them readily one after the other. They were also freely eaten by insectivorous Birds. The experiments strongly support the view that the gilded appearance is of protective significance.—Sept. 9, 1887. E. B. P.

In order to test my suggestions as far as possible, I communicated with different naturalists in various parts of the world.

Mr. ROLAND TRIMEN kindly sent me the following letter from the Cape:—

“As regards gilded butterfly pupæ, I am unfavourably placed here for observation of them.

“We have only *P. cardui* and *Danaïs chrysippus* at this end of the country. The former varies much in the amount of gilding on the back and wing-covers, but I think that the three rows of dorsal tubercles are always brightly gilded. This larva does not seem particular as to site of suspension, apparently hanging itself indiscriminately to plants, walls, fences, etc.; but I have found it more frequently on walls, especially under the coping.

“The latter (*D. chrysippus*) has only a half-girdle (dorso-abdominal) of contiguous golden dots—set off by an immediately preceding black tuberculated ridge—and eight scattered golden spots about the head and thorax. This pupa is either green or pinkish, and sometimes of a tint combining both those colours. It is usually suspended to its food-plant.

“‘I have not found the variable colouring of this pupa to accord with its immediate environment, though I have allowed the larvæ in confinement free choice of various convenient surfaces for pupation, with the view of ascertaining whether there was any relation between the green or reddish tint and the colouring of adjacent objects. It seems not improbable that this brilliant pupa stands in no need of special protection, but, like the imago (and apparently the larva also), is avoided by insectivorous animals.’—Extract from my forthcoming work, ‘South African Butterflies.’

“Other Danaine pupæ appear to be exceedingly brilliant. Thus THWAITES (MOORE’S ‘Lepidoptera of Ceylon,’ p. 2) says: ‘The suspended chrysalids’ (of the Danainæ) ‘are brilliantly metallic in colouring’; and BOISDUVAL (‘Faune Ent. de Madag.’ etc., pp. 36, 37) describes thus the pupa of *Euplœa goudotii*: ‘La chrysalide ressemble à une bulle d’or extrêmement brillante’; and thus that of *Danaïs phœdon*: ‘La chrysalide est . . . d’un vert doré brillant.’

“I should incline to the view that in this protected (distasteful) group of the Danainæ the conspicuously brilliant colouring of the pupæ is a warning signal of ‘Not fit to eat!’

“Is it possible that the gilding in pupæ of other (not distasteful) Butterflies may originally have been acquired as protective mimicry of the brilliant Danaine ones?

“The only other South-African gilded Butterfly pupæ that I know of are those of the genus *Atella* (nearly allied to *Argynnis*). In these two species (which I only know by drawings and descriptions) the gilding (which in one is rather *silvery* than golden) is confined to spots on head and thorax, narrow borders of wing-covers, and dorso-abdominal raised spots bearing either pointed tubercles or thin spines. I have no information as to the objects to which these pupæ are usually attached.

“The pupæ of the Acraeinæ (which, as you know, are a protected group of Butterflies), though (as far as I know) never gilded, are yet exceedingly conspicuous, their

ground-colour being white or yellowish, veined or streaked with black, and marked abdominally with bold orange-and-black spots, orange tubercles, or even (*Planema aganice*) long pink filaments!

“Very splendid gilding (evanescent after death) occurs in some of the Tortoise-beetles (Cassididæ). In these insects—almost circular in outline, flat abdominally, convex superiorly, with subdiaphanous extended margin—which rest exposed on leaves, there certainly is much resemblance to glittering dew-drops.”

The evidence that *P. cardui* seems to prefer mineral surroundings is favourable. The Danainæ are evidently the group of pupæ which make use of this appearance as a warning, but, for the reasons given above, I think it is more probable that the protective colouring is ancestral, and its use as a warning is secondary, rather than that the development of the appearance took place in the reverse order. The fact that Mr. MANSSEL WEALE proved the existence of colour susceptibility in the pupa of *Acræa ezebria*—one of a group protected by the possession of an unpleasant taste—greatly supports the suggestion that the conspicuous warning appearance has been secondarily acquired.

It is very probable that, the gilded appearance having been once acquired, and closely resembling a very widespread and conspicuous mineral, the continued use of a small proportion of the colouring might be protective when the insect no longer pupated on mineral surfaces, because of the fact that the appearance does suggest the common mineral, whatever the position of the insect may be, and because of the resemblance to the glittering of dew upon leaves, as Mr. TRIMEN suggests. Thus the amount of gilding present upon the green form of *V. Io* may, perhaps, have this protective value.

Dr. FRITZ MULLER kindly wrote to me on the subject from Brazil as follows:—

“Leider kann ich Ihnen über goldige oder mit goldenen Flecken gezielte Puppen nur sehr wenig sagen; nur zwei derselben habe ich im Freien angetroffen: die von *Mechanitis lysimnia* und die von *Danaïs erippus*. Beide gehören zu der ungeniessbaren Gruppe der Danainen, und es ist der Goldglanz wohl ein Warnungszeichen. Die Raupe von *Mechanitis lysimnia* lebt an mehreren stacheligen *Solanum*-arten in kleinen Gesellschaften, und an der Unterseite der Blätter der Futterpflanze hängen sich die Puppen auf; es gibt nichts Prächtigeres als diese ganz und gar im schönsten Metallglanze strahlenden, nicht selten zu 10, 12, oder mehr beisammenhängenden Puppen. Die Raupe von *Danaïs erippus* lebt an *Asclepias curassavica*; die Puppe habe ich nie an der Futterpflanze gesehen, dagegen oft an Bretterzäunen u. dgl. aufgehängt. Die Puppe ist grün und nur mit einigen goldenen Punkten geziert, von denen eine quere Reihe kleiner lebhaft glänzender Wärzchen am meisten in die Augen fällt.

“Andere Puppen mit Metallschimmer, der aber nie zu so hellem Goldglanz wird wie bei *Mechanitis*, habe ich nur in der Gefangenschaft gesehen, z. B., von verschiedenen *Adelpha*-arten. Ich glaube, dass hier der Metallglanz nicht als Warnung vor Ungeniessbarkeit, sondern als Schutz dient, weiss aber nicht in welcher Weise. Da

in unserem Urwalde Gestein nirgends zu Tage tritt, ist bei diesen grossentheils an Urwaldsbaumen lebenden Arten wohl kaum an Aehnlichkeit mit mineralischen Substanzen zu denken. Alle Puppen, die ich im Freien an Pflanzen zwischen Laub gefunden habe (z. B., *Morpho*, *Caligo*, *Prepona*, *Siderone*, *Catanephele*), sind grün, mit Ausnahme von *Acræa* (ungeniessbar), deren Puppen weiss sind, mit schwarzen Domen.

“Mit dem Bedauern, dass ich über Ihre Ansichten in Betreff der goldigen Puppen, die mir sehr annehmbar scheinen, so gut wie nichts aus eigener Erfahrung sagen kann, &c.”

The use of the gilded appearance as a warning in *Mechanitis* seems to be very clear, and the fact that the pupæ hang in companies must of course greatly add to the effect, and this is probably the meaning of the habit (so common, for this reason, among many distasteful and conspicuous larvæ). It seems probable that the gilded spots of *Adelpha* are instances of persistence with protective value among changed surroundings and withdrawal from the substance originally imitated. From Dr. MÜLLER's letter it is quite clear that the resemblance to mineral substances cannot be of any protective importance in the Brazilian forests, but it does not therefore follow that such surroundings would not intensify the appearance even now. Thus *V. urticæ* in England has been shown to be rendered very brilliant by gilt surroundings, although in nature it can rarely receive such a stimulus, and the amount of gilding on it is very small or entirely absent. It is satisfactory that Dr. MÜLLER should consider my suggestion a probable one as to the biological significance of the gilded appearance.

My friend and pupil, Mr. E. A. MINCHIN, who has collected insects and observed very keenly in India for many years, informs me that the pupa of *Euplæa core* is exceedingly conspicuous, being covered with a brilliant metallic silvery appearance, and hanging from its food-plant in such a manner that it can be seen at a great distance. As the butterfly is exceedingly common, and is known to be protected by disagreeable properties, it is almost certain that the metallic appearance of the pupa acts as a warning. This, however, was the only instance of the use of this colour for such a purpose which had come under the observation of Mr. MINCHIN.

In conclusion, the balance of evidence given by experiment and observation is, I think, in favour of the view that the metallic appearance was originally of protective value from its resemblance to glittering minerals; that it has preserved its original significance amid change of surroundings; while in other cases it has come to be used for an entirely different purpose to render distasteful forms conspicuous.

Experiments upon Papilio machaon.

I received 11 almost mature larvæ from Mr. W. H. HARWOOD, of Colchester, on August 24, 1886. The following experiments were made. The eight largest larvæ

were selected August 24, and divided into two lots of four each, so that the sizes of the larvæ in the two lots were as equal as possible. Each lot was placed in a clear glass cylinder (of about 8 centimetres in internal diameter and 1·8 decimetre in height) with a roof and floor of brown paper. The food-plant (fennel) was confined to the lowest part of the cylinder and was very small in amount, and it was removed directly the larvæ ceased feeding, while the rest of the cylinder was completely filled with dead and brown twigs of a coniferous shrub and the dry brown stems of *Aconitum napellus* with the brown seed-vessels adherent.

The four larvæ in one cylinder were carefully blinded August 24, a process which could be very effectually carried out in this species, for the larvæ are very quiet, and, furthermore, all the ocelli are placed upon a distinct black patch; and when the latter is covered with an opaque varnish the ocelli must be also covered. On August 25 the larvæ were painted with varnish a second time, and on the 26th a third time. The other larvæ remained normal.

August 26, 9.20-P.M. One of the blinded larvæ had changed into a green pupa on the brown floor, but in close proximity to the green leaves of the food-plant, and one of the normal larvæ had also changed into a green pupa, which was fixed to one of the brown coniferous twigs; but here also the green food-plant was just beneath. The food-plant was then removed from both cylinders, as the larvæ had become full-fed.

In each cylinder four green pupæ were obtained, fixed to the brown stems or roof, or lying free on the floor. (One of these is figured in Plate 26, fig. 14.) These results surprised me very much, as I knew that there was a well-marked brown variety of the pupa not uncommon in this species.

Of the remaining larvæ two were placed (August 25) in a smaller glass cylinder (about 6 centimetres in internal diameter and 1 decimetre in height) covered with a single layer of green tissue-paper, and with a roof and floor of the same material, and with abundant food-plant.

The last larva was placed under exactly similar conditions, but was blinded (renewed as above).

This last larva and one of the former died, but the remaining larva pupated upon the green food-plant, and to my great surprise it produced a *distinct brown variety*. (This pupa is represented in fig. 15, Plate 26.) At first sight these results appear to be extremely startling, especially when it is considered that the experiments in this paper were directed towards the investigation of an adjustable imitative resemblance which, when present, forms the highest culmination of this method of protection. But, in the first place, it was probable that the green cylinder was more shaded than the other, and hence the production of the brown form might be accounted for, the species being simply susceptible to comparative darkness or illumination, and producing its corresponding dark or light variety in obedience to the respective stimuli; although the experiments showed that the ocelli have nothing to do with the susceptibility. Furthermore, the eight green pupæ were produced from the largest and

healthiest larvæ, while the remaining three were small, and of these only one lived to pupate. Hence an unhealthy condition, or even a stunted size, might become secondarily associated with one of the two varieties of a dimorphic species after the power of being influenced by the surroundings had been lost. Nevertheless, it is not necessary for the growth of some such association that the loss of susceptibility should have taken place, for it has already been shown that the gilded appearance and corresponding absence of pigment colours are associated with the presence of parasites in the pupæ of the *Vanessidæ*. It is clear from my experiments, and the previously quoted experience of others, that the susceptibility to corresponding colour influences has been lost in *P. machaon*, and Dr. FRITZ MÜLLER shows that it has also been lost in *P. polydamus*. On the other hand, the striking observations of Mrs. BARBER upon *P. nireus*, and of Mr. TRIMEN upon *P. demoleus*, prove conclusively that these species are highly susceptible to the influence of certain colours; and when it is remembered that both of the former non-sensitive species are dimorphic, and furthermore present in each case the two varieties, green and brown, which harmonise best with their surroundings, it appears probable that such dimorphism is the remnant of a former susceptibility which has, at any rate to a great extent, disappeared. Future experiments must finally decide whether the relative amounts of illumination produce any effect upon *P. machaon*, as Mr. HARWOOD believes, or whether either form of pupa exhibits a more or less constant relation to a healthy or unhealthy condition, or finally, whether the formation of either variety is the spontaneous result of individual variability.

Experiments upon Pieris brassicæ and P. rapæ.

Having read of Mr. T. W. WOOD's observations, and those of other naturalists, I was extremely anxious to obtain the larvæ of these species to investigate in the manner already described under *V. urticæ*, &c. I could only obtain single specimens in Oxford, and I thought that the experiments would have to be delayed for another season. However, just when the experiments on *V. urticæ* came to an end, on September 8, I went to Seaview, in the Isle of Wight, and there found the kitchen gardens ravaged by the larvæ of *P. brassicæ*, while those of *P. rapæ* were very abundant on mignonette. Accordingly, I made the experiments described below, and, although they were not as accurately or minutely conducted as those on *V. urticæ*, they yield some valuable results, and entirely confirm the previously adopted conclusion that the colour-effects are due to larval and not to pupal sensitiveness. Being away from my laboratory, and not expecting such an opportunity, it was, of course, impossible to carry out the experiments in the most satisfactory manner. The results obtained with the two species are described together, because they were, in nearly all cases, kept under similar conditions and were, in fact, often placed in the same cylinders. Furthermore, the results were remarkably uniform. When no locality is mentioned in any experiment it is understood that the included larvæ were captured at Seaview.

Both species are peculiarly adapted for experiments of this kind because of their quiet disposition and the great length of the period preparatory to pupation.

Before describing and classifying the results of the experiments upon the larvæ of *P. brassicæ*, it is necessary to construct a standard of the various degrees of colour assumed by the pupæ. In making such a list, it is necessary to take account of two features, each of which varies—the ground-colour, and the pigment patches and spots which are visible to the naked eye. Seven of the principal varieties are figured in Plate 26, figs. 24—30, all $\times 2$.

(1) The normal form. In these pupæ the ground-colour is always more or less greyish from the abundance and relative size of minute black pigment spots which occupy depressions in the cuticle. They can often be distinguished with the naked eye on close and careful inspection; but their general effect is to produce the greyish appearance. The large black pigment patches and spots are nearly always abundant, and when the ground-colour is darkest the former also contribute towards a dark appearance by their especial size and number. The ground-colour may be of various tints—greyish-green, orange, yellow, or a peculiarly opaque-looking greyish-white. The amount of the grey colour always present subdues the differences between these tints, so that they resemble each other far more than the above description would seem to imply. The wings and under-side are always lighter than the rest of the surface, especially as regards the ground-colour, for the pigment patches are often very pronounced in these parts of the pupa. The following subdivisions are well marked, although transitional varieties occur:—

- (α) The darkest forms with greyish-green, orange, yellow, or white ground-colour.
- (β) Intermediate forms, with lighter ground-colour of the same tints, and smaller and fewer pigment patches.
- (γ) The lightest of these forms, with ground-colour still greyish, but the pigment patches very small relatively to (α) or (β).

(2) The last subdivision passes into this variety, in which the ground-colour is an opaque-looking whitish-yellow, often with greenish areas on part of the surface, the pigment patches being very small. The greyish hue is lost because of the minute size of the dots in the ground-colour. Hence the effect is very light. The wings and under-side are lightest, and not so opaque as the dorsal and lateral surfaces; the pigment spots in these parts are small.

(3) A still more abnormal, very well-marked, variety, possesses a deep transparent-looking bluish-green ground-colour, in which the minute dots and the large patches are even less developed than in the last degree. An opaque whitish-yellow band, like the ground-colour in (2), occupies the anterior half of that part of the third abdominal segment which is seen dorsally, and extends on to the posterior part of the segment in front; and the dorsal surfaces of the abdominal segments behind the third are often mottled with the same colour. The median dorsal ridge is strongly marked in orange, interrupted in the abdominal region by opaque greenish-white, and the

supra-spiracular ridge and line are of this latter colour. The wings and under-side are pale transparent yellowish-white, with very small pigment patches.

The differences between the ground-colours of (1), (2), and (3) are very well-marked, whereas the predominant grey often masks the differences between the ground-colours of (α), (β), and (γ).

It is similarly necessary to construct a standard of the colours met with in *P. rapæ*. The colours of 10 of the chief varieties are figured in Plate 26, figs. 32-41, $\times 2$, and in fig. 31, natural size.

(1) The darkest forms are plentifully dusted with minute black dots, producing a very dark grey appearance. There is very much pigment on the wings, and black patches are especially developed on the dorsal and sub-dorsal ridges or lines, and upon the rostrum. The ground-colour is hardly recognisable apart from the grey dusting, but can be seen clearly in certain parts of some pupæ, and is then usually of a faint pinkish or dull yellowish tint, or some mixture of these colours.

(2) Much less dark, due to the reduction in the amount of the minute dots and the black patches, which occur in the positions described above. Nevertheless, these pupæ are, as a rule, of darkish-grey appearance. The ground-colour is often more clearly recognisable, and is generally of the same tints as above, but the differences between the various tints are not generally well-marked until (4) is reached.

(3) Still lighter, but with sufficient of the grey dusting to obscure the tint of the ground-colour and to produce a grey or light-grey appearance. The black patches still occur in the same positions, but they are smaller; the same ground-colours are recognisable.

(4) Very light, with little or almost none of the grey dusting, so that the ground-colour is predominant in producing the general appearance. The black spots and patches are very slightly developed, and sometimes entirely absent, except for a few black points on the side of the rostrum, which is the last position in which traces of the pigment patches are retained. It is, however, common to find a slight, but distinct, speckling due to minute black points, but not sufficiently numerous to combine with the lighter ground-tint and produce a grey result. The ground-colours are much more distinct, as they are not dimmed, and are generally pinkish, yellowish, or faint greenish, or some combination of these. The latter colour is transitional into the brighter tints of the next degree.

(5) In certain pupæ the green ground-colour is sufficiently distinct to warrant their classification as a separate degree. All varieties of colour are met with, from the faint, scarcely perceptible, yellowish-green tinge of certain pupæ in the last degree to the more distinct and bright yellow greens arranged under this head, and finally up to a magnificent transparent emerald-green, which forms the culmination of the development of this tint as a ground-colour. There are also dull greens, and sometimes these pupæ are dusted with grey spots and have the black markings developed to a

considerable extent (such a pupa is figured Plate 26, fig. 31, natural size), but, as a rule, these pupæ are the lightest of all in both these respects. The lens, however, shows the existence of minute dots in all cases, although in the more extreme forms very few minute points can be detected by the naked eye, and there is no trace of the black markings even upon the rostrum. It is very common in the extreme forms of this degree, and in the lightest of the last degree, for the median and lateral ridges and the extremities of the body to be of a distinct pink tinge.

SERIES 1.—LARVÆ SUBJECTED TO VARIOUS COLOURS.

I. *Black.*

A.

On September 13, 10 mature larvæ of *P. brassicæ* and four of *P. rapæ* were placed in a cylinder 7 centimetres in internal diameter and 18 centimetres in height, lined entirely with thick blackened paper, and with a roof and floor of the same material. Thus the glass was entirely concealed, and the larvæ were everywhere surrounded by a black background, while light was almost completely excluded. In the following Tables the larvæ of *Pieris brassicæ* are indicated by the letters P. B., and those of *Pieris rapæ* by P. R. Periodical inspection gave the following data for estimating the length of the period preparatory to pupation, &c. :—

| | | |
|---------------------|--|--|
| Sept. 13, 9.30 A.M. | Experiment began. | |
| " 15, evening | 2 larvæ, P. B., girdled | Stage III. at least 20-30 hours in these cases. |
| " 16, 6.15 P.M. | 1 P. B. pupated, and 2 girdled; 1 P. R. girdled | About 70 hours for the whole preparatory period of the 1 pupated. |
| " 17, 7.30 P.M. | 2 P. B. pupated, and 1 P. R.; 7 P. B. girdled | Between 90 and 100 hours for the whole period of the 2 pupated. |
| " 18, 7.50 P.M. | 6 P. B. and 1 P. R. pupated; 3 P. B. and 1 P. R. girdled | Over 100 hours for the whole period of some of these; others may not have begun Stage I. directly they were put in the cylinder. |
| " 19, 8.47 P.M. | 7 P. B. and 2 P. R. pupated. | |

Results.—Except when it is otherwise stated, the pupæ were all compared together on January 23-26, 1887, and, as nearly the whole of them were thus placed together, the results are very trustworthy.

Eight pupæ of *P. brassicæ* were alive when the comparison took place.

5 were fairly crowded on the roof; of these. . . 2 were (1), α , the ground-colour almost entirely grey.
3 " (1), β , 1 with a yellowish-green tinge, and
2 faintly orange.

3 were scattered over the side, towards the upper part, but not crowded 1 was (1), α , with a faint orange tinge.
2 were (1), β , both greenish.
3 G 2

4 living pupæ of *P. rapæ* were similarly compared.

1 was with the group of 5 *P. brassicæ* described

above, and it was a light (3), with a yellowish-grey ground-colour and a rather small amount of grey dusting.

3 were scattered over the side with the 3 *P.*

brassicæ, and of these 2 were (3), typical, and of the usual light-grey colour caused by the abundant sprinkling of minute dots over the light ground-colour, which is apparently very pale pinkish in this case, but very hard to determine.

1 was (4), with very little pigment and a dim yellowish ground-colour.

The effect of the dark surroundings is thus much more manifest in the pupæ of *P. brassicæ* than in *P. rapæ*; nevertheless, the latter are very different from those produced in white surroundings. It is very strange that the results of a deep black surface exposed to daylight should be darker than those produced by the same surface in darkness, and yet this appears to be the case, for the pupal colours on the tarred fences are much darker than those described above. At the same time, the pupæ on the fence were generally shaded in cracks and corners, and the fence itself was in a shady lane, but the amount of light must have been in all cases far larger than in the cylinder. Such results are the reverse of those obtained in the case of *Vanessa urticæ*.

B.

September 11, at 7.30 P.M., 10 nearly mature larvæ of *P. rapæ*, found on mignonette at Seaview, were placed in a cylinder (6.7 centimetres in internal diameter and 2.24 decimetres in height) lined for rather more than half of its internal circumference with opaque blackened paper, and with a roof and floor of the same material. Some of the food-plant was also included. No notes were taken as to the times of pupation, &c.

Results.—10 pupæ were obtained, of which—

5 were on the black roof, and of these. 1 was (2), a little lighter than usual; yellowish-pink.
4 were (3), 1 considerably lighter than normal and greenish; 3 normal and pinkish.

4 were on the clear side of the cylinder, but so high up that they came against the inflected edge of the roof, and of these

3 „ (1), not very black for this stage; 1 distinctly pinkish, the others chiefly grey.

1 was (3), rather lighter than normal, and yellowish-pink ground-colour.

1 was fixed on to the background near to the roof, and it was a

(1), typical pinkish ground-colour as far as it could be seen.

These pupæ are decidedly darker than those of the preceding subdivision, and yet they were freely exposed to light: on the other hand, they are less dark than the wild larvæ found on the tarred fences, which, although shaded (in my own observations), were probably in a stronger light than that to which the pupæ of this subdivision were exposed. These results seem to show that, at any rate in *P. rapæ*, the stronger illumination of a black surface tends towards the production of stronger effects, just as would be the case with a white or green surface, while the direct white light falling on the larval surface produces no antagonistic effects.

C.

September 15. Four pupæ were found at this date upon a black tarred fence in a shaded lane at Seaview, and they were compared with the other pupæ examined on this date. All were (1), very dark; two pinkish, and two so dark that the ground-colour could hardly be made out, but probably yellowish. These results are very uniform, and show the influence to have been very strong. It has already been mentioned that the pupæ were in most cases concealed in angles and corners, &c., and this was also true of the succeeding pupæ.

D.

October 5.—Three pupæ of *P. rapæ* were found October 5 on a black tarred fence in a shaded lane at Seaview. All were (1); one very black, one normal, and one rather light for this degree and distinctly pinkish: the others being very dark grey, so that the ground-colour was almost entirely concealed, but apparently pinkish in one pupa and yellowish-pink in the other. These results are very uniform and highly protective.

E.

Mr. W. H. HARWOOD kindly sent me 11 pupæ of *P. rapæ* found upon tarred palings, and all of these were (1); five extremely black, two normal, and one very grey rather than black; three dead, but apparently very dark as far as could be ascertained from the large amount of pigment on the pupal wings. The ground-colour seemed to be pinkish in all cases. These results are exceedingly uniform, and show the very strong effect of the black surface.

In concluding the effects of black, it will be of interest to give a tabular analysis of the effects of this background, in various degrees of illumination, upon *P. rapæ*:—

| Degrees of colour. | Dark (1) | (1) | (2) | (3) | (4) | (5) |
|---|----------|-----|-----|-----|-----|-----|
| I. A. Black background in darkness. . . | .. | .. | .. | 3 | 1 | .. |
| " B. " " somewhat shaded . . . | .. | 4 | 1 | 5 | .. | .. |
| " C. } " " less shaded . . . { | 4 | .. | .. | .. | .. | .. |
| " D. } " " " " " " " " { | 1 | 2 | .. | .. | .. | .. |
| " E. " " " " " " " " { | 5 | 6 | . | .. | .. | .. |

This analysis seems to prove almost conclusively that the stronger illumination increases the influence of the black surface upon the larvæ. At the same time, it must be remembered that A. and B. were larvæ which had been kept for some time in the cylinders, whereas C., D., and E. had been under more normal conditions, as the pupæ were found wild.

The relation of the effect of black surfaces to those of other colours will be shown later in an analysis of the colours of all pupæ which had been exposed to such surroundings during the whole of the preparatory period.

II. *White.*

A. *In almost complete darkness.*

On September 9 a large number of mature larvæ of *P. brassicæ* and a few of *P. rapæ* were placed in a light-blue cardboard box, of which the internal surface was white. (The length was 3 decimetres, width 1·1 decimetre, and the depth 7·7 centimetres.) The cardboard was thick and opaque, so that light could only enter to a slight extent between the lid and the box, and perhaps at the angles. The larvæ were thus exposed to a white surface in almost complete darkness. No notes were taken as to the times of pupation, &c.

Results.—When the comparison took place there were 41 living pupæ of *P. brassicæ*, and, of these, 20 were fixed to the roof, being greatly crowded in an irregular group 1 decimetre long and 7 centimetres in its greatest width.

Of these 20 pupæ 4 were (1), α , very dark indeed; 3 being very faintly orange, and 1 almost entirely grey, but slightly greenish.

14 „ (1), β , 5 being faintly orange, and the others more or less yellowish-green, but the grey tints predominant in all.

2 „ (1), γ , the ground-colour yellowish-green, mottled with deeper green. Black patches small.

2 pupæ were fixed to the roof close together, but at a distance from those described above. 1 was (1), β , faintly orange.

1 „ (1), γ , distinctly greenish and less grey than usual; the black patches rather more developed than usual.

1 pupa was isolated on the roof, and was (1), γ , yellowish, mottled with greenish; typical.

3 pupæ had been previously taken off the roof, or had fallen off it; position unknown 2 were (1), β , pale greyish-orange ground-colour; 1 of them with a rather deeper tint, and mottled with greenish.

1 was (1), γ , greyish-green; typical black patches.

15 pupæ were crowded on one end of the box and on the adjacent part of one side, but not at a greater distance than 4 centimetres from the end. None were close to the bottom of the box, but nearly all were crowded along the top of the end and side

Of the 15 pupæ, 2 were (1), α , very dark; 1 faintly orange, the other greenish-yellow.

13 „ (1), β , 5 greenish, 2 very pale ochreous. 2 yellowish, 2 faintly orange, 2 yellowish-green. All except the first five were rather light for this degree, both as to the amount of grey dusting and the large black patches, but they were not light enough for (1), γ .

There were only two pupæ of *P. rapæ*, of which one was in the group of 20 *P. brassicæ* described above, and it was a (4), with very little pigment and a pale-yellowish ground-colour. The other pupa was isolated in one of the corners where two of the sides met, opposite to the end where the 15 pupæ of *P. brassicæ* were crowded. It was (2), typical, with the ground-colour almost entirely grey, but apparently faintly yellowish also. Hence it is clear that the white surroundings had produced very little effect upon the larvæ, which, indeed, was to be expected, considering the almost complete darkness. There is no doubt, however, that the pupæ of *P. brassicæ* are not as dark as those formed in the dark (I., A.) upon a black surface, and hence some effect seems to have been caused by the exceedingly feeble amount of light which penetrated. The two pupæ of *P. rapæ* differed widely, one being the form commonly occurring on white or light surfaces, the other much darker.

B. *Very strongly illuminated.*

September 11, 9.30 P.M.—Many larvæ of *P. rapæ* were placed in a cardboard box (2.1 decimetres in length, 1.22 decimetre in width, and 8.5 centimetres in depth) which was lined with white glazed paper, and with a clear glass front, directed, as in the other cases, towards a strong light and close to a north-east window. The larvæ, some of which were apparently mature, had been found upon mignonette at Seaview, and some of this food-plant was also included. No notes were taken as to the times of pupation.

Results.—19 living pupæ were obtained in this experiment, and of these—

3 were fixed to the glass front, near together,

and near to the white sides of the box;

and of these. 2 were (4), very little pigment, 1 with a pale-pinkish, the other a yellowish-pink ground-colour.
1 was (5), almost no pigment; a pale, but bright, yellowish-green ground-colour; the ridges and extremities of the body pale pink, as is common in green varieties.

13 pupæ were thickly crowded along the angles

made by the roof and adjacent parts of the

two sides with the back, and of these . . . 2 were (3), very light for this degree, 1 pinkish and the other yellowish

10 „ (4), very little pigment on all but 1, and this 1 but little more than normal; 4 pinkish, 4 yellowish-pink, and 2 yellowish-white.

1 was (5), very little pigment; pale, but distinct, yellowish-green ground-colour, with pink tips and ridges.

3 pupæ were isolated on the roof, sides, &c ;

of these 2 were (4), both pinkish: 1 typical, 1 with very little pigment.

1 was (5), little more pigment than usual; very pale yellowish-green ground-colour.

The strong effect of the white back-ground in producing light-coloured pupæ is well seen in the above descriptions. It is noteworthy that the degree of colour represented by (4) harmonises far better with this background than that represented by (5). Hence the (5) were of a very pale-greenish colour, and not conspicuous as well-marked green varieties would have been. Furthermore, the (4) were in nearly all cases very deficient in pigment, so that in this respect they were quite equal to normal (5).

C. *Strongly illuminated, but not equal to B.*

A few larvæ of *P. rapæ* were made use of in some experiments to test, by blinding, whether the influence of surrounding colours acts through the ocelli. Two glass cylinders of equal size (8.2 centimetres in internal diameter and 1.81 decimetre in height) were covered externally with a single layer of white tissue-paper, and with a roof of white glazed paper, and a floor of ordinary white paper. The cylinders were placed in a fairly strong light, several feet from a north-east window. The experiment was conducted as follows:—

| Dates. | α. Blinded. | β. Normal. |
|---------------------|--|---|
| Sept. 17, 10 P.M. . | Experiment began | Experiment began. |
| " 18, 8.40 P.M. . | 1 larva, which had been found in Stage I. or II. on a plain wood fence at Bembridge, was girdled; another, still feeding, having been found on mignonette at Seaview | 2 larvæ had both gone up the side and were sitting motionless; food removed; they had been found on mignonette at Seaview. Another, added at this time, found in Stage II. under the cement coping of a gate pillar at Spring Vale. |
| " 19, 9.5 P.M. . | No further change | 2 larvæ mentioned above were girdled. |

No further notes were taken, but all five larvæ produced living pupæ.

Results.—(α) Of the two pupæ, one was fixed to the roof and the other horizontally to the side just below : both were (3), but light for this degree : one distinctly pinkish, and one faintly yellowish-green, the latter very small and dwarfed.

(β) Of the three pupæ, one was fixed to the roof, one about halfway up the side, and one near the bottom of the side ; all were (4), one faintly pinkish, one yellowish and dark, intermediate between this degree and (3), and one yellowish and much dwarfed. The pigment patches and dots were normal in two of the pupæ.

Although the two blinded larvæ produced rather darker pupæ, the differences were *very* slight indeed, and are quite insufficient to support the conclusion that the ocelli represent the part of the larva which is sensitive to these influences. The larvæ remain quiet when blinded, like those of *P. michuon*, and are very well suited for this method of investigation. The effects of white surroundings are shown in the pupal colours.

D. *Strongly illuminated, but not equal to B.*

A little later another similar experiment was made, the same cylinders being used. Four larvæ found on mignonette were placed in one cylinder, and three blinded larvæ, also found on mignonette, were placed in the other. No notes were taken as to suspension, &c. Seven pupæ were obtained.

Results.—Of the four pupæ which were produced from the normal larvæ—

- | | |
|---|---|
| 1 was fixed to the roof, and was a | (4), normal, brownish-pink ground-colour. |
| 3 were fixed horizontally to the side just beneath the roof, and of these | 2 were (4), normal, both very faintly pinkish. |
| | 1 was (5), of a pale dull green and more grey than usual, and more of the pigment patches, so that, as far as pigment is concerned, it would have been a somewhat dark (4). |

Of the three pupæ which were produced from the blinded larvæ—

- | | |
|--|---|
| 1 was fixed to the roof, and was a | (4), yellowish, with rather more of the pigment patches than usual ; grey dusting normal. |
| 1 was fixed horizontally to the side, just beneath the roof, and was a | (4), very pale yellowish-pink, with an almost complete absence of pigment, except upon the rostrum ; so also very little grey dusting. Altogether, considerably lighter than normal. |
| 1 was fixed horizontally about halfway down the side, and it was a | (3), with a distinct dull-greenish ground-colour ; it much resembled the (5) of the normal pupæ, only the grey dusting predominated, so that the green was largely concealed. Dusting normal for (3), black patches rather less than usual. |

These results negative the view that the ocelli represent the sensitive organs sought for, for the two sets of pupæ were, on the whole, as equal as possible. The latter set of three pupæ included one lighter than any of the other set, and two somewhat darker; but the differences were slight in all cases. The larval heads remained in proximity to their respective pupæ, so that the success of the blinding could be tested afterwards, and the varnish, when examined with a lens, appeared to completely cover the ocelli and the surrounding area.

The light pupæ produced by the white surfaces are well seen in the 12 pupæ of these experiments.

It will now be interesting to give a tabular analysis of these results, showing the effects of white surfaces with different degrees of illumination in the case of *P. rapæ*.

| Degrees of colour | (1) | (2) | Dark (3) | (3) | Light (3) | Dark (4) | (4) | Light (4) | Pale (5) | Deep (5) | |
|---|-----|-----|-------------|-----|--------------|-------------|-----|--------------|-------------|-------------|------|
| II., A. White surface in almost complete darkness | .. | 1 | .. | .. | .. | .. | .. | 1 | . | . | = 2 |
| " C } White surface in strong | .. | .. | . | .. | 2 | 1 | 2 | .. | .. | .. | = 5 |
| " D. } illumination | .. | .. | .. | 1 | .. | 1 | 3 | 1 | 1 | .. | = 7 |
| " B. White surface in very strong illumination | .. | .. | .. | .. | 2 | 1 | 1 | 12 | 3 | .. | = 19 |
| Total | .. | .. | .. | . | .. | | | .. | . | .. | 33 |

This analysis shows well the increasing effect produced by increasing illumination, for the description of the apparatus used in C. and D., on the one hand, and in B. on the other, indicates that the differences between their degrees of illumination must have been very great. Furthermore, the background of B. was entirely composed of an intensely white and strongly reflecting surface, while in C. and D. the roof (and to some extent the floor) alone possessed this property to a considerable extent, the sides of the cylinder being composed of white tissue-paper. On the other hand, there was a large amount of food-plant which remained until after pupation had taken place in B., while only a small quantity was placed in the cylinders, and this was removed when the larvæ had ceased to feed.

III. *Red*.

A.

On September 13, 15 mature larvæ of *P. brassicae*, and the next day many larvæ of *P. rapæ*, were placed in a cylinder 8.2 centimetres in internal diameter and 17.8 centimetres in height, lined internally with deep-red opaque paper for about three-quarters of its circumference, and with a roof and floor of the same substance. The experiment was conducted as follows. This and the following colours made use of in these experiments are shown on Plate 26, figs. 16-21 inclusive.

| | | |
|---------------------|---|--|
| Sept. 13, 9 15 A.M. | Experiment began. | |
| " 14, 8 45 P.M. | Many larvæ of <i>P. R.</i> added. | |
| " 15, EVENING | Many <i>P. B.</i> girdled | Stage III. at least 20-30 hours in these cases. |
| " 16, 6.25 P.M. | 2 <i>P. B.</i> pupated, and about 9 girdled; 1 <i>P. R.</i> girdled | About 70 hours for the whole period of these 2 larvæ. |
| " 17, 7.35 P.M. | 6 <i>P. B.</i> pupated (2 of them deformed): 1 <i>P. R.</i> pupated; 6 <i>P. B.</i> girdled | About 100 hours for the whole period of some of these; others may not have begun Stage I. at once. |
| " 18, 8.35 P.M. | 11 <i>P. B.</i> pupated, and 1 <i>P. R.</i> pupated; 1 <i>P. B.</i> and 1 <i>P. R.</i> girdled | |
| " 19, 9.10 P.M. | 12 <i>P. B.</i> pupated, and 2 <i>P. R.</i> pupated. | |

Results.—11 pupæ of *P. brassicæ* were alive on January 23, when the comparison was made, and—

Of these, 7 were rather crowded upon the roof.

Of the 7 4 were (1), α , very grey and dark, but 2 of them showing a faint orange tinge, and the other 2 apparently greenish, but the grey is entirely predominant.

2 " (1), β , greyish-white, mottled with green.

1 was (1), γ , greyish-green, with opaque whitish marks as in (3) degree of colour. This form is transitional into (3), retaining the greyish tinge of (1), although subdued so that the green is well-marked, while the pigment spots are small for (1).

2 were isolated on the clear glass front of the cylinder, and of these . . .

1 was (1), α , very dark; faint orange tinge.

1 " (1), β , greyish-green, with some white.

2 were isolated on the red background, fixed in a horizontal position just beneath the roof, and of these both .

were (1), β , 1 being faintly yellowish, the other apparently yellowish-green, but with the grey predominant.

Hence the red background produced very dark results in the case of *P. brassicæ*, for out of the 11 pupæ there is only a single (1), γ , while there are five of the darkest forms (1), α . The pupæ of *P. rapæ* were all dead.

B.

Mr. HARWOOD also sent me 35 pupæ of *P. rapæ* found upon red brick walls.

Of these, 21 were (1), 12 very dark indeed, and apparently with a dull-pinkish ground-colour; 9 normal, with the same tinge.

2 " (4), very grey, but with hardly any black patches; a yellowish and a pinkish variety.

12 pupæ were dead, and, as far as I could judge, they were mostly very dark varieties.

Hence the pupæ were as a whole extremely dark, like those of *P. brassicæ*. It should be noted that many of them may have been upon the mortar, or in dark corners, under coping, &c., and doubtless the red colour of the bricks was very variable, depending upon the age of the walls.

Since writing the above, I have had an experience which confirms my hesitation in accepting the above results as necessarily following from the red colour. I had long noticed a large number of pupæ of *P. rapæ* upon Keble College chapel, and when I removed them in order to describe the colours for this paper I carefully observed and noted the places of pupation. After I had collected 20 or 30 pupæ I found it useless to proceed, as they had almost without exception been attacked by *Ichnemon* flies and were dead and faded. I noticed, however, that not a single pupa was attached to the red brick wall of the chapel, but to the mortar immediately beneath the projecting stone-courses, or upon the overhanging surfaces or hollows of the stone itself.

IV. Orange.

A.

On September 14, 12 mature larvæ of *P. brassicæ* and 10 of *P. rapæ* were placed in a cylinder 8 centimetres in internal diameter and 18 centimetres in height, and lined internally with deep-orange opaque paper for about two-thirds of the circumference, and with a roof and floor of the same material. Periodical examination produced the following results:—

| | | |
|-------------------|--|---|
| Sept. 14, 10 A.M. | Experiment began. | The whole period for the 5 pupated must have been less than 56 hours if it began when they were placed in the cylinder. |
| " 16, 6.5 P.M. | 4 P. B. pupated, and 1 P. R.; 8 P. B. girdled, and 3 P. R. girdled | |
| " 17, 7.25 P.M. | 7 P. B. and 2 P. R. pupated; 5 P. B. and 3 P. R. girdled. | |
| " 18, 8.35 P.M. | 12 P. B. and 4 P. R. pupated; 1 P. R. girdled. | |
| " 19, 9.15 P.M. | 12 P. B. and 5 P. R. pupated; 2 P. R. girdled. | |

Results—All the 12 pupæ of *P. brassicæ* were alive when they were compared, and of these eight were rather crowded on the roof.

Of the 8 pupæ 2 were (1), β , both yellowish, and not so grey as usual in this degree.

6 " (3), very typical forms.

1 pupa was fixed in a horizontal position on the clear glass side, but so high up that it came against the orange background formed by the margin of the paper forming the roof. It was

(3), an unusually deep bluish-green on almost the whole of the dorsal surface.

1 pupa was fixed horizontally on the clear glass just below the last. It was . . .

(3), typical.

1 pupa was fixed vertically on the clear glass about $\frac{1}{2}$ from the roof. It was . .

(2), with rather larger black spots than usual.

1 pupa was fixed vertically on the orange background, but high up and close to the roof. It was

(3), typical.

The five living pupæ of *P. rapæ* were also compared at the same time, and—

Of these, 2 were fixed horizontally on the clear glass side, but high up, so that they came against the orange background formed by the margin of the paper roof (as above).

The two pupæ were both .

(5), with a pale transparent yellowish-green ground-colour: very little pigment on 1 pupa, and perhaps rather more than usual on the other.

1 was fixed to the roof with the *P. brassicæ* described above. It was a

(4), typical, with a distinct, but very pale, yellowish-green ground-colour.

1 was fixed vertically to the clear glass about $\frac{1}{2}$ from the roof, and it was a

(4), typical; very faintly yellowish ground-colour.

1 was fixed on the orange background close to the roof, and it was a . . .

(5), a beautiful bright-yellow green, with hardly any pigment.

The strong effects of the orange background in the prevention of pigment formation and in the production of a green ground-colour are very interesting and remarkable, for the results, although so well-marked, are certainly not especially protective. It is also most interesting that *P. brassicæ* and *P. rapæ* should have been influenced so uniformly. The bright green variety of *P. brassicæ* chiefly formed by the use of this background is represented in Plate 26, figs. 29 and 30, $\times 2$.

B.

About October 13 a few larvæ of *P. brassicæ*, which, I believe, were found upon *Tropæolum*; and a few *P. rapæ*, were placed in a cylinder lined with orange paper of the same size and arrangement as that already described. No notes were taken as to the dates of pupation.

Results.—Two pupæ of *P. brassicæ* were obtained :

Of these, 1 was isolated on the clear glass front,

and was (1), β , normal; somewhat pale ochreous ground-colour.

1 was isolated on the background, very

low down; it was (1), γ , rather unusually greyish for this degree, but deep-green anteriorly and opaque-looking whitish-green posteriorly; the usual small black patches.

1 pupa of *P. rapæ* was isolated upon the clear glass side, and it was a (4), rather dark for this degree, and possessing a dull-yellowish ground-colour.

The same results of the orange background are seen in these pupæ, but they are not equal to those described in Division A.

V. *Yellow.*

A.

On September 14 four mature larvæ of *P. brassicæ* and four of *P. rapæ* were placed in a cylinder 6 centimetres in internal diameter and 10 centimetres in height, lined internally with light-yellow opaque paper for about three-fourths of its circumference, and with a roof and floor of the same colour. Periodical inspection gave the following results:—

| | | |
|----------------------|---|--|
| Sept. 14, 10.25 A.M. | Experiment began. | Whole period less than 56 hrs if it began when they were placed in the cylinder. |
| " 15, 10.25 P.M. | 2 P. B. girdled for at least 12 hrs, as they were so in the morning when inspected. | |
| " 16, 6 30 P.M. | 2 P. B. pupated lately, and 1 girdled, and also 1 P. R. | |
| " 17, 7.40 P.M. | 3 P. B. pupated and 1 P. R. girdled. | |
| " 18, 8.30 P.M. | 3 P. B. and 1 P. R. pupated, and 2 P. R. and 1 P. B. girdled. | |
| " 19, 9.13 P.M. | 4 P. B. pupated and 2 P. R. pupated; 1 P. R. girdled. | |

Results.—Three pupæ of *P. brassicæ* were alive when all were examined and compared:—

- 1 on the roof was (1), γ, typical; yellowish, with the faint greyish tinge and small black patches.
 2 were scattered over the side, and of these 1 was (2), typical.
 1 „ (3) „

Two living pupæ of *P. rapæ* were similarly compared,

- 1 being on the roof; it was a (5), with a distinct, but dull-green, ground-colour, much dusted with grey dots, and an amount of pigment on ridges, rostrum, &c., which would be typical of (3).

The other pupa was isolated on the clear side of

- the cylinder, and it was a (3), with a rather distinct yellowish ground-colour, and rather less grey than usual in this degree.

Thus the effects of yellow appear to be in the same direction as those of orange, although, as far as the evidence goes, the influence does not appear to be equally strong.

VI. *Green.*

A.

September 14.—Four larvæ of *P. brassicæ* and one of *P. rapæ*, and later four more of the former and four of the latter, were placed in a small glass cylinder (6 centimetres in internal diameter and 8.5 centimetres in height) completely covered externally with one layer of green tissue-paper, and with a roof and floor of the same material. Notes were taken at the following dates:—

| | | |
|---------------------|--|--|
| Sept. 14, 9.15 P.M. | Experiment began. | |
| " 15, EVENING | 1 P. B. girdled | |
| " 16, 7 P.M. | 4 P. B. girdled, and the P. R. pupated some time | The P. R. must have passed at least 4 hrs in the period if it began when larva was placed in the cylinder. |
| " 17, 7.25 P.M. | 4 more P. B. and 4 P. R. added | |
| " 18, 8.30 P.M. | 3 P. B. pupated, and 1 P. R., 3 P. B. and 1 P. R. girdled | Under 72 hrs. in the period if it began with the experiment |
| " 19, 8.55 P.M. | 6 P. B. pupated, and 1 P. R.; 2 P. B. and 1 P. R. girdled | Under 48 hrs. for some of these if the period began with the experiment. |
| " 19, 8.55 P.M. | 6 P. B. pupated, and 2 P. R.; 2 P. B. girdled | |

Results.—Seven living pupæ of *P. brassicæ* were obtained :—

6 were crowded on the side near the roof,

and of these 5 were (2), all with the characteristic opaque yellowish ground-colour, mottled with green, and small black patches and spots. One was yellower than the others, with less of the green mottling.

1 was (3), rather greyer-green than usual, and rather larger black patches.

1 pupa was more isolated, and an outlying

member of the group described above: it was (1), β , with a rather brighter and more distinct yellowish-green ground-colour than usual, because the grey was less pronounced.

Two living pupæ of *P. rapæ* were similarly compared, both being in the group of six *P. brassicæ* described above; and

Of these, 1 was a (3), light for this degree, with a faint yellowish-green ground-colour, and much less of the grey dusting than usual posteriorly; normal anteriorly.

1 was a (4), with very little pigment and very pale pinkish ground-colour.

It is very remarkable that the green surroundings should have shown less influence than orange or yellow in the production of varieties which by their colour are especially protected upon the first-named colour. It must be remembered, however, that the conditions of experiment were different, tissue-paper being used in this case, and highly illuminated opaque reflecting surfaces in the former. Nevertheless, tissue-paper has been shown to produce marked effects with *V. Io*, and it certainly made a bright green background. And here, although the effects seem small when compared with those of orange, they are in reality considerable, and are all in a protective direction.

B.

September 15.—10 larvæ of *P. brassicæ* and 13 of *P. rapæ* were placed in a glass cylinder (7.5 centimetres in internal diameter and 17.25 centimetres in height) completely covered externally with one layer of green tissue-paper, and with a roof and floor of the same material. The *P. rapæ* had been found upon mignonette, and were mostly full-fed, but after September 16 they were fed upon cabbage. The following notes were made :—

| | | |
|---------------------|---|---|
| Sept. 15, EVENING . | Experiment began. | |
| " 16, 7 P.M. | 5 P. B. girdled | |
| " 17, 7.30 P.M. | 2 P. B. pupated, 6 girdled; 2 P. R. girdled | Under 48 hours' period for these 2 if it began with the experiment. |
| " 18, 7.52 P.M. | 8 P. B. pupated, 1 girdled; 1 P. R. pupated, and 2 P. R. girdled | So also under 72 hours for these additional ones. |
| " 19, 8.52 P.M. | 9 P. B. pupated, 1 P. R. pupated; 3 P. R. girdled, and 1 P. R. making girdle. | |

Results.—Nine pupæ of *P. brassica* were obtained, and—

- Of these, 1 was on the roof, and was (1), β , black patches normal, dusting rather less, and ground-colour rather more distinct than usual; yellowish-green, inclining to very pale orange in places.
- 7 were on the side, fairly crowded, and all upon the upper $\frac{1}{4}$ of the height of the cylinder; of these 2 were (1), β , 1 pale-greenish and 1 very pale orange; dusting less than usual; black spots normal on former, less than normal on latter.
- 4 were (2), almost normal, but the ground-colour a little greener than usual.
- 1 was (3), typical.
- 1 was more isolated on the side, and was . . . (2), with normal ground-colour, but the black spots more developed than usual.

Eight living pupæ of *P. rapæ* were similarly compared, and—

- Of these, 2 were on the roof, of which 1 was a (5), very little pigment, and very pale greenish ground-colour; and
- 1 „ (4), very little pigment, and a pale yellowish-pink ground-colour.
- 1 pupa was on the side near the top, in the crowd of 7 *P. brassica* described above, and it was a (4), typical, with a greyish-yellow ground-colour.
- 5 pupæ were more isolated on the side, and of these 2 were (3), but both light for this degree, 1 with a yellowish-pink ground-colour, and 1 with a dull, but distinct, greenish ground-colour.
- 2 were (4), 1 typical, 1 with hardly any pigment; the ground-colour faintly yellowish-pink in both.
- 1 was (5), of a distinct green ground-colour, but dull for this degree, and with the greyiness and black patches of a light (3).

These results are very uniform with those of Division A. The more numerous *P. rapæ* in this division show that the green surroundings have some considerable effect, as one-fourth of the pupæ were the green form (5). The bright yellowish-green variety of *P. brassicæ*, which was chiefly formed by the use of this background, is represented in Plate 26, fig. 28, $\times 2$.

C.

September 13.—20 larvæ of *P. brassicæ* and 10 of *P. rapæ* were placed in a shallow wooden box (2.26 decimetres long, 1.3 decimetre wide, and 5.2 centimetres deep: all internal dimensions) lined internally with thick paper tinted with a pale bluish-green colour, which became whiter owing to the pigment being removed in many places. The box was made to stand on one of its long sides and covered with a sheet of clear glass, and it was placed close to the window, so that a strong north-east light was directed into the interior. Notes were taken at the following hours:—

| | | |
|---------------------|---|--|
| Sept. 13, 9.50 P.M. | Experiment began. | |
| " 16, 6.5 P.M. | 1 P. B. pupated; 5 girdled | The box was inclined backwards at an angle of about 45°, so that the roof was illuminated to an extent equal to that of the rest of the box, for the larvæ tended to collect on the roof for pupation. |
| " 17, 8.15 P.M. | 4 P. B. pupated, 8 girdled; 3 P. R. girdled | Under 72 hours for the 1 pupated, if the period began with the experiment. |
| " 18, 9.15 P.M. | 7 P. B. pupated, 7 girdled; 3 P. R. pupated and 2 P. R. girdled. | Under 95 hours for these additional ones. |
| " 19, 9.25 P.M. | 10 P. B. pupated, 2 girdled; 3 P. R. pupated and 2 P. R. girdled. | |

Results.—11 living pupæ of *P. brassicæ* were obtained.

7 were crowded in a corner of the roof,

and all were (1), β , with normal black patches, but the ground-colour a peculiar greenish-white, very opaque-looking, much dusted with grey, as usual: with a slight orange tinge in 1 pupa, and 2 pupæ rather more yellowish-green than the others.

1 pupa was isolated on another part of the roof, and

was (1), β , like the others, and especially resembling the 2 last described.

3 pupæ were isolated on the clear glass front, and of these

2 were (1), α , one of them faintly orange, the other pale greenish, rather unusually distinct, because the grey dusting, although very pronounced, was locally collected into bands and patches, leaving the colour comparatively undimmed between them.

1 was (1), β , resembling the 3 last described of this degree, only a brighter colour than any others, because of the comparative absence of the grey dusting: a bright yellowish-green.

Three living pupæ of *P. rapæ* were similarly compared, and—

Of these, 1 was isolated on the side of

the box, and it was a (4), pink ground-colour, and very little pigment.

2 were isolated on the glass

front, and of these . . . 2 were (3), one typical, with a yellowish ground-colour; the other very light for this degree, with a pinkish ground-colour.

The influences in this division seem to have been much weaker as far as the prevention of pigment formation is concerned than in the two preceding experiments, and this is probably on account of the colour employed. The green, although bright, was of a very bluish delicate tint, and was easily removed or rendered paler. On the other hand, there was a special peculiarity about the ground-colour of the majority of the pupæ of *P. brassicæ* (one of which is represented in Plate 26, fig. 27, $\times 2$) which harmonised well with these surroundings, and appeared to indicate a power of special colour adaptation to minute differences or peculiarities in the surroundings which was not seen to an equal extent in any of the other experiments.

D.

A mature larva of *P. rapæ* was found upon cabbage in a garden at Oxford about the beginning of September, and was placed in a small cylinder covered with one layer of green tissue-paper, and with a roof and floor of the same substance. Mignonette was introduced as the food-plant, but the larva, without feeding, pupated upon one of the leaves. The pupa was a deep green (5), but with an unusual development of the black markings for this stage. The markings were present to the same extent as in a typical (3), although the grey dusting was very deficient. The distinct and bright ground-colour left no doubt of the real degree of the pupa. This pupa is represented in Plate 26, fig. 31, natural size.

E.

October 5, a large number of pupæ were found on a very deep-green gate in a shaded lane at Seaview, but the vast majority contained the larvæ of parasitic Hymenoptera, and had dried up when the comparison was made.

It is also to be noticed that the pupæ were nearly always concealed in the shadows of the mouldings and under the overhanging parts of the framework, &c.

Only 6 pupæ remained alive when the comparison took place.

Of these, 1 was (1), typical, yellowish-pink as far as the ground-colour could be seen through the abundant pigment.

2 were (3), 1 typical and apparently faintly yellowish-pink, the other light for this degree and distinctly pinkish.

3 „ (4), all about normal, and yellowish-pink.

There must have been quite 20 more which had died, and there certainly was not a (5) among them, for I remember being astonished at the time at the small effect produced by the green background. One *P. brassicæ* was found at the same time, which also died from the same cause, but it was a distinct (1), and probably the common (1), β .

It is probable that these surprising results are due to the peculiarly deep and strong colour of the paint, against which the usual bright transparent yellowish-green, or the delicate emerald-green of the (5) degree of colour, would have been at least as conspicuous as the darker varieties which were actually found. There was certainly a great contrast between the colour of the gate and the more delicate green tints used in the preceding experiments.

VII. *Blue.*

A.

September 11, 8.45 P.M. 10 nearly mature larvæ of *P. rapæ* were placed in a cardboard tray (1.72 decimetre in length, 8.4 centimetres in width, and 2.7 centimetres in depth) lined internally with dark-blue paper, and with a clear glass front, which was directed towards a strong north-east light, the tray being placed vertically close to the window. A small amount of the food-plant (mignonette) on which the larvæ were found at Seaview was also included in the tray. No notes were taken as to the times of pupation.

Results.—Eight living pupæ were obtained, and of these 5 were crowded together in the angle made by two of the sides, one of which formed the roof in the position in which the tray was placed.

Of the 5 pupæ 3 were (3), 1 with more of the pigment patches than usual, although less of the grey dusting, and with a yellowish-pink ground-colour. The others lighter than usual for this degree, and with a pinkish ground-colour.

2 „ (4), 1 typical, with a very pale pinkish ground-colour, and 1 with very little pigment and a pale yellowish-green ground-colour.

3 pupæ were isolated on the sides,

and of these 2 were (3), typical, with a yellowish-pink ground-colour as far as it could be seen through the abundant grey dusting.

1 was (4), with a distinct pinkish ground-colour, and rather more pigment than usual for this degree.

It is quite clear that the blue background was without special effect on the larvæ, acting merely as a moderately dark surface in abundant white light, with results intermediate between those of a black and of a white background.

Now that I have given the results of experiments in which all the colours of the spectrum have been used except violet, it will be well to make a tabular analysis of the whole, with the view of testing the relative effects of the colours, and of investigating the effect upon pigment of the predominance of rays of any particular wavelength in the light incident upon the larval surface, or of combinations which produce a corresponding effect on the Vertebrate eye.

These results are extremely interesting, especially as the effects of different-coloured light are so similar in the two species, both as to the formation of pigment and the production of green or other ground-colours. The effects in the former case are so uniform, and are so graduated in the successive colours which were used, that it is possible to give an approximate representation of the results by a graphic method making the abscissæ of the scale of wave-lengths, and each ordinate of a length which corresponds to the average amounts of pigment obtained from all the pupæ subjected to any one colour. Of course the results are only approximate, for there must be a good deal that is arbitrary in the selection of the scale of lengths to correspond to the different amounts of pigment in each degree of colour. The scales which were made use of in obtaining the averages were as follows:—

| <i>P. rapæ</i> | | | <i>P. brassica.</i> | | |
|----------------|------------------|--------------|---------------------|--|--------------|
| | | millimetres. | | | millimetres. |
| Light | (5) { Deep green | 5 | (8) | | 14 |
| | (4) { Pale " } | | (2) | | 28 |
| | (4) | | (1), γ | | 42 |
| Dark | (4) | 10 | (1), β | | 56 |
| Light | (3) | 15 | (1), α | | 70 |
| | (3) | 20 | | | |
| Dark | (3) | 25 | | | |
| | (3) | 30 | | | |
| | (2) | 40 | | | |
| | (1) | 50 | | | |
| Dark | (1) | 70 | | | |

In order to decide upon the points on the scale from which to draw the ordinates, the colours employed in the above-described experiments were examined with the spectroscope, and hence a test of their purity was also obtained. The tints are indicated on Plate 26, figs. 16–21. The results were as follows:—

Dark-red opaque paper.—The reflected light was brightest from 60–65 on the scale below, the rest of the spectrum being very dim indeed, and even the chief reflected rays were not very bright. Hence the darkness of the tint.

Deep-orange opaque paper.—The reflected light was brightest from 57–65, the yellow being especially bright; the rest of the spectrum was very dim indeed. The chief reflected rays were much brighter than above, and the colour of the paper was far more brilliant.

Pale-yellow opaque paper.—The reflected light included a large part of the spectrum from 51–65, which was very bright, while the blue was reflected to a much less extent, and there was some more complete absorption between the blue and the green.

Bright-green tissue-paper.—The transmitted light (which was almost exactly the same as the reflected light, differing only in intensity) which had passed through one layer of this paper was especially dim in the blue, and in the red to a less extent, the

green passing through with hardly any diminution. The transmitted rays were especially bright from 51-59.

The pale bluish-green opaque paper made use of in Experiment VI., C. The reflected light differed from that transmitted through the green tissue-paper in the much larger amount of blue rays which entered into its composition. The red, orange, and yellow were also reflected to a slight extent, but were chiefly absorbed. The chief reflected rays extend from 45 to 57 on the scale given below.

The dark-blue opaque paper.—The chief reflected rays extended from 44 to 48, the rest of the spectrum being very dim. As the chief rays were not intense, the colour was a very dark blue.

It is, therefore, seen that the colours employed were fairly pure, although, of course, they were all mixed with an immense proportion of white light. The spectroscopic results indicate that each of the colours should be made into separate ordinates—the green tissue-paper and the pale bluish-green being separated because of the different amounts of blue in their composition. The red brick walls (III., B.) are omitted because of the reasons given at the end of the description of the pupæ found upon them, and because the spectroscopic character of their colour is unknown; and for the latter reason the greens in VI., D., and VI., E., are also omitted.

In the graphic representation (fig. 6) given below each ordinate which starts from the part of the base line which represents the visible spectrum is made to diverge and include on the scale the limits of the rays which were shown by the spectrum to constitute, at any rate, the chief part of the colour transmitted or reflected by the colour indicated on the ordinate. It should be noted that the *P. rapæ* line merely passes the ordinate for dark red, for there was no experiment to indicate the amount of pigment which is formed under the influence of this colour in the case of *P. rapæ*. To the left of the red end of the spectrum four ordinates are added to represent black and white under three conditions of illumination, in order to compare their results with those produced by certain parts of the visible spectrum.

The actual lengths were as follows:—

| | <i>P. rapæ.</i> | <i>P. brassica.</i> |
|-----------------------------------|-----------------|---------------------|
| | millims. | millims. |
| White in very strong light . . . | 7.36 | |
| White in light | 12.91 | |
| White in nearly complete darkness | 22.5 | 56.84 |
| Black | 47.81 | 61.25 |
| Red | .. | 61.09 |
| Orange | 8.38 | 26.0 |
| Yellow | 12.5 | 28.0 |
| Green | 10.5 | 33.25 |
| Pale bluish-green | 16.66 | 58.54 |
| Blue | 18.75 | |

The results in the case of *P. brassica* are probably more trustworthy, because the numbers of pupæ employed in the various experiments were more uniformly large. The similarity of the results in the two species is very striking, and it would have been even greater but for the fact that the palest varieties of *P. brassica* retain more pigment than those of *P. rapæ*. Looking at the colours which retard the formation of pigment when they preponderate in the incident light, we see that they contain certain rays in common which are probably highly efficient in this respect, i.e., the rays from 57 to 59 or 60. The immense difference between the action of red and orange corresponds to the fact that these active rays are present in the latter, and they are also present in the highly efficient yellow and bright green—that is, in all

Fig. 6



the colours which retard the formation of pigment, except white, which, of course, contains these rays in addition to the others, although this is not necessarily the case, and it would be extremely interesting to experiment with whites from which this part of the spectrum is absent. Concerning such an experiment, it may be argued that this relation of colour to pigment formation is essentially protective, and is, therefore, concerned with the visual perception of insect-eating animals, and especially the Vertebrata; and if these latter cannot with the unassisted eye distinguish between a pure and an impure colour, or between a white which contains all the colours of the spectrum and one which contains only some of them, it would seem that the pupa would lose immensely if it were influenced by the one and not by the other, or in different directions by the two. It is, however, clear that we must only expect perfect parallelism between the sensitiveness of such widely separated animals as far as the stimulus is provided by colours which form the natural environment of the pupæ.

The observations upon the length of the preparatory period and of its constituent stages were insufficient, but they indicate a much greater length than in the case of *V. urticae*. In most cases the period began when the larvæ were placed in the cylinders, &c., for no food was eaten by the majority of larvæ after they had been captured. This was because I always selected the largest larvæ from the cabbages and, as was previously explained, the shock of capture hastens the beginning of the period when the organisms are practically mature.

It is also noteworthy that there are some indications, as in the case of *V. urticae*, that darkness may act in such a way as to prolong the whole period, and that possibly this increased length of time may bear upon the formation of pigment; or, conversely, that a shortened period may be brought about by certain reflected colours, and that the absence of pigment may ensue as a secondary result. This suggestion appears to be worth a careful trial, and, even if it does not contribute to the elucidation of this most difficult question, the protracted period in darkness may be useful to the organism in another way—to give it the opportunity of being affected by surrounding colours after change in the conditions of illumination. Thus, if the most sensitive part of the period were passed during the night, it would be to the advantage of the species for such a susceptible condition to be prolonged as far as possible. It may be that the absence or presence of direct light may be important in this respect, but the whole subject needs careful experimental investigation along the lines suggested by the results of the experiments described above.

SERIES 2.—PUPÆ FOUND UPON VARIOUS SURFACES OF MIXED OR INDEFINITE COLOURS.

A certain number of wild pupæ were found upon walls, cement, &c., of colours which were not distinct enough to be included in the last series; and a large number of captured larvæ were allowed to pupate without any precautions to ensure uniformity in the colour of surrounding surfaces.

The results are described below :—

I. Mr. HARWOOD also sent me two pupæ of *P. rapæ*, found upon a cemented wall. They were both (3), with a distinct pinkish ground-colour, and one of them with unusual development of black patches on the dorsal surface generally, without any special development on the ridges and keel. These colours would be of protective value against the greyish surface.

II. September 20. Two pupæ of *P. rapæ* were found fixed to some yellowish-grey brick gate pillars, but shaded under the coping, at Yarbridge (Isle of Wight).

Of these 1 was (3), pinkish, lighter than usual.

1 " (4), pinkish, unusually grey, but with a smaller amount of pigment patches than usual.

The pupæ were well protected, for their colour harmonised with that of the bricks, &c.

III. October 12. A single pupa of *P. brassicae* was found shaded under the coping of some new yellow brickwork at Seaview, Isle of Wight, in a somewhat shaded situation. It was a (1), β , typical grey dusting, pale-greenish ground-colour; normal black patches.

At the same time and place a number of pupæ of *P. rapæ* were found, nearly all of which were shaded by the coping of the brickwork. When the comparison took place eight pupæ were alive, and

Of these 1 was (2), typical; yellowish
 4 were (3), typical; faintly pinkish.
 3 .. (4). all yellowish-pink; 1 with very small amount of pigment, the others with more than usual.

These pupæ also were well protected. The colour of the bricks was not sufficiently uniform or strong to be included under the yellow of the last series, and it was also complicated by the new whitish mortar and the new stone coping.

IV. September 15. A number of pupæ of *P. rapæ* were compared at this date, having been chiefly obtained from larvæ found upon mignonette in my garden at Oxford, while a few were produced from larvæ taken from the same food-plant at Seaview. The larvæ had been kept in two glass cylinders (about 8 ½ centimetres in internal diameter and 1·8 decimetre in height), the tops being covered with white muslin, somewhat dull and grey from age, while the cylinders stood upon white plates and were almost filled with abundant food-plant. 31 pupæ were obtained, of which

7 were fixed to the two muslin tops, and of

these 1 was (1), pinkish.
 4 were (4), 2 yellowish-green, 1 pinkish, 1 greenish.
 2 „ (5): both very pale yellowish-green, with a little pigment like that on (4) and in the usual positions.

19 pupæ were fixed to the glass of the two cylinders, and of these

2 were (1), 1 pinkish, 1 yellowish-green
 6 „ (2) and (3), for these had not been separated at this time, the classification being extended later: 3 were pinkish, and 3 yellowish-green.
 9 were (4): 6 pinkish, 3 of them very faint and pale; 2 greenish, and 1 yellowish-green.
 2 „ (5). 1 very extreme and of a splendid emerald-green, with an almost complete absence of pigment in any form; the other pale yellowish-green, also with very little pigment.

5 pupæ were loose in the cylinders, and all 5 were (4), 1 pinkish and 4 greenish.

These results, upon the whole, show well the effects of white and green surroundings in strong illumination.

SERIES 3.—TRANSFERENCE EXPERIMENTS.

A few transference experiments were made with the larvæ of these two species, and, although on a far smaller scale than with *V. urticae*, the results prove quite conclusively (1) that the larva and not the pupa is sensitive to the colour of surrounding surfaces; (2) that there is some susceptibility to such influences during Stage III.

I. *Gilt, black, and white during Stage III.*—September 11. On this date seven larvæ of those described above in the two cylinders (see Series 2, IV.) were girdled on the glass sides, and small pieces of black, white, and gilt paper were glued under each to test the effect of these backgrounds during the remainder of Stage III. Two pieces of paper were placed under each in such a manner that the method of suspension was not interfered with, and yet a complete background was obtained. The experiment was conducted as follows:—

| Sept. 11, 9.30 P.M. to 9.50 P.M. | Gilt paper fixed under 2 larvæ | White paper fixed under 3 larvæ | Black paper fixed under 2 larvæ | Larva girdled on stem of migno- nette, pinned against gilt back- ground. |
|-------------------------------------|---|--|---|--|
| Sept. 12, 8.30 A.M. | Both pupated . . . Results: Both (4); 1 pinkish and 1 greenish; very little pigment | 1 pupated . . . Results: A (4), pale pinkish; a good deal of pigment for this stage | 1 pupated . . . Results: A (4), yellowish-green; very little pig- ment | Pupated Results: A (2) or (3) then com- bined; yellowish- green |
| " 12, 9.50 A.M. | .. | .. | 1 pupated; hence 12 hours of Stage III spent on the black. Results: (4), yel- lowish-green with normal pigment. | |
| " 12, 7.30 P.M. | . | 1 has pupated; perhaps about 18 hours of Stage III on the white paper. Results: (4), pale pinkish; very little pigment | | |
| " 12, 9.45 P.M. | . | No further change | | |
| " 13, 8.40 A.M. | . | The last larva has pupated; at least 24 hours on the white paper, and probably 30 hours. Results: (4), pink- ish; normal pig- ment. | | |

The pupæ were compared with those above, September 15.

In these pupæ there is no evidence that any effect was produced by the back-grounds during Stage III, or part of it. The results should be compared with those of Series 2, IV., which show the colours of the pupæ which had not been subjected to transference.

II. *Black and white during Stage III.*—September 16, a small group of larvæ of *P. brassicæ* were girdled upon a clear glass sheet which formed the covering of an ordinary wooden box. Other larvæ and freshly-formed pupæ fell down from the same sheet, and will be described below (III.). A sheet of white paper was fixed under half of the group and a black sheet under the other half, and a shelf was fixed between the two colours, covered with white paper towards the white side and black towards the black side, and the whole was placed vertically in a strong north-east light, close to the window. Thus the two groups of larvæ were exposed to black and white respectively during the remainder of Stage III., and there was reason to believe that the stage had not long begun. Notes were taken as follows:—

| | | |
|------------------|--|--|
| Sept. 16, 7 P.M. | Experiment began, the paper and shelf having been fixed. | |
| " 17, 7.20 P.M. | 1 larva on the white area has only just pupated | 24 hours on white; thus Stage III. longer than this period of time. |
| " 18, 8.30 A.M. | Another larva on the white area has pupated a few hours | It is a (2), opaque greenish-white, rather yellow anteriorly; normal. |
| | And 3 larvæ on the black area have pupated a few hours | It is a (2), opaque greenish-white, lighter than the above, and smaller black spots. |
| | And 1 larva on the black area was now pupating | 2 alive, and both intermediate between (1), γ , and (2), because of the amount of grey dusting; very small black patches. |
| " 18, 11.40 A.M. | The last larva on the black has pupated | Dead. Stage III. at least $37\frac{1}{2}$ hours. |
| | | Dead. Stage III. longer than $37\frac{1}{2}$ hours |

It thus appears that the pupæ were slightly affected by the white and black surfaces, although to a very small extent. Stage III. may have been somewhat protracted in consequence of the glass being turned in such a position that the larvæ were head downwards, an attitude *never* assumed in this species before pupation.

III. *Black and white during Stage III.*—At 7.30 P.M., September 16, six moist and freshly-formed pupæ and seven girdled larvæ of *P. brassicæ* (alluded to above) fell down from a clear glass sheet to which they had been suspended (being the covering of an ordinary wooden box), owing to the continuous silken web which they had spun becoming detached from the glass. This was thought to be an opportunity of testing the sensitiveness of the pupa to coloured surroundings (as opposed to the larva), and therefore the pupæ and larvæ were each divided into two groups, and were placed on a black and a white surface. By 7.5 P.M., September 17, six of the larvæ had pupated, and the seventh pupated at 9.15 P.M. on the same evening.

Results.—Seven pupæ were living which had been exposed to the black surface:

Of these, 4 were (1), β , 3 yellowish-green, but very grey; 1 greenish.

3 " (1), γ , ground-colour opaque whitish-yellow, almost like (2), but with larger black patches than in this degree, and even more developed than is usual in (1), γ .

Four pupæ were living which had been on the white surface :

Of these, all 4 were (1), β , 2 an opaque whitish-green, 1 yellowish, and 1 chiefly grey, but with a greenish tinge ; the grey very marked in all of them.

Here, again, the surfaces may have produced some slight effect, but the conclusions are not certain, because the pupæ already formed when the experiment began became intermixed with those which had been exposed to these colours during Stage III.

IV. On September 15 (see Series 1, I, C.) a girdled larva of *P. rapæ* (Stage III.) was found on a black tarred fence and was removed (4.15 P.M.), and was placed on white paper. At 5.30 P.M. on the next day it had pupated an hour or two, and the pupa when it took the permanent tint became a (1), very dark, and like those which had been found upon the fence on the 15th. In this case the powerful effects of the black surface during Stages I. and II. and the first part of III. could not be altered by the exposure to a white surface during the remainder (24 hrs.) of the last stage. This experiment is very conclusive against the former theory of pupal sensitiveness.

V. October 12, a single larva of *P. rapæ* in Stage I. was found crawling upon a chocolate-coloured paling at Seaview ; it was placed in a small chip-box with the other pupæ found on this date (see Series 2, III.) and became a (4), about normal, with a yellowish tinge. In this case the pupal colour appears to have been entirely due to the influence of the light-yellowish tint of the chip-box in which both the terminal stages were passed.

VI. October 5, six larvæ of *P. rapæ* were found on the green gate (described in Series 1, VI., E.), of which three were in Stages I. or II., and three in Stage III. (girdled). All six were placed in a cylinder (6 centimetres in internal diameter and 1.05 decimetre in height) covered with a single layer of black tissue-paper, and with a roof and floor of the same material. All the six pupæ which were obtained were alive when the comparison was made.

Of the 3 pupæ which had passed Stage III.

in the cylinder and were girdled, 1 was (3), typical, yellowish.

2 were (4), 1 typical and yellowish-pink, and 1 was faintly greenish and very deficient in pigment.

The 3 pupæ which had passed part of

stage in the cylinder were all (4), typical, 1 pinkish, 1 yellowish-pink, and 1 yellowish.

Here, again, some slight effect appears to have been produced by the transference, and the results harmonise well with those of all the other experiments of the kind.

In concluding the account of experiments upon these pupæ, it must be remarked that the effect of the coloured surroundings upon the dark pigment is, perhaps, the least important part of the changes produced, for there are other consequences which

seem to be much deeper in significance and far more difficult to understand. The black pigment patches and minute black dots are cuticular and superficial, while the ground-colours are sub-cuticular and deep-seated; and in the most brightly coloured pupæ they are mixed colours due to the existence of different pigmentary (and probably chlorophylloid) bodies present in the different elements and at different depths of the sub-cuticular tissues of the same pupa. In other pupæ no trace of such colours can be seen. Hence we see in these most complex and varied effects of the stimulus provided by the reflected light, which deepen into their permanent pupal condition very many hours after the stimulus has ceased to act, the strongest evidence for the existence of a chain of physiological processes almost unparalleled in intricacy and difficulty, while a theory of comparatively simple and direct photo-chemical changes induced by the stimulus itself without the intervention of such a physiological circle seems entirely inadequate as an explanation of the facts.

Observations upon the Colours of the Pupæ in the Genus Ephyra.

After the consideration of the variable pupæ of many species of Rhopalocera it is interesting to compare the results obtained after an examination of the equally exposed and variable pupæ of a single genus of the Heterocera—the genus *Ephyra*. In 1833 I had the opportunity of studying the life-histories of three species of this genus (*E. pendularia*, *E. omicronaria*, and *E. orbicularia*), and an account of the investigation is published in the ‘Transactions of the Entomological Society of London,’ Pt. I., 1834, pp. 50–56. A short summary of the results obtained is given below. The larvæ of *E. pendularia* are dimorphic in the last stage, appearing in the two most usual colours, green and brown; those of *E. omicronaria* are similarly dimorphic, but the brown forms are relatively rare; while the larvæ of *E. orbicularia* are variable. The dimorphism of the two former species extends into the pupal stage, the brown larvæ always becoming brown pupæ, and the green larvæ green pupæ. (The two forms of *E. omicronaria* are shown on Plate 26, figs. 22 and 23, natural size.) Hence the colour of the pupa can only be affected through the influences which determine the larval colour, and it has not yet been shown that the colours of these larvæ can be controlled, although, from many experiments on other larvæ, I think that the proof of such a relation to surrounding colours is likely to be afforded by experiment.

The pupal and larval dimorphism has no relation to sex or to any observable imaginal character. Statistics appeared to prove that the brown forms of *E. pendularia* (alone observed in sufficient numbers) are relatively abundant in the winter (larvæ and) pupæ, and green in the (larvæ and) pupæ of the summer broods. It was also shown that the relative preponderance of either form could be greatly increased by breeding from parents which possessed the same colour in the earlier stages. Observations upon the situations selected for pupation failed to establish any colour-relation; but the results were not convincing against the existence of such a relation,

for the experiment was not carried out in the best way: there was not a sufficient quantity of *both* colours in the surroundings.

Dr. WILHELM MÜLLER, of Greifswald, refers to the above account, and evidently regards the *Ephyridæ* as peculiar in this respect. His evidence is all the more valuable because of his careful work on larvæ and pupæ of Lepidoptera during many years spent in South America. He says ("Sudamerikanische Nymphalidenraupen," SPENGLER, 'Zoologische Jahrbücher,' vol. 1, Jena, 1886, p. 234):—"Eine ruhmliche Ausnahme machte E. B. POULTON, welcher feststellt, dass sich bei verschiedenen Species der Gattung *Ephyra* der Dimorphismus der Raupe bei der Puppe erhält, so dass helle Raupen nur helle Puppen, dunkle Raupen nur dunkle Puppen liefern." The colours of the pupæ being predetermined, and following rigidly the colours of the respective larvæ, it follows that these organisms afford an interesting contrast to all the other species of exposed pupæ described in this paper (for there is no colour-relation between the larvæ and pupæ in *Papilio machaon*, &c.), while special protective resemblances in the pupæ seem to be only possible as the results of the selection of appropriate colours upon which to pupate. In the above-recorded observations there was quite insufficient evidence to support the theory that the larvæ have any such power, but I do not think that they are by any means conclusive in the other direction.

SUMMARY.

The results of this paper may be shortly summarised as follows:—

1. The following exposed pupæ of the Rhopalocera have been proved in this paper to possess an adjustable colour-relation to their surroundings—*Vanessa Io*, *V. urticae*, *V. atalanta*, *Pieris brassicae*, and *P. rapæ*. The relation had been previously proved for some of these species, and for others, which I have not had an opportunity of investigating, e.g., *Papilio nireus* and *P. demoleus*.
2. On the other hand, dimorphic pupæ which are closely allied to the sensitive forms may be uninfluenced by surrounding colours, e.g., *Papilio machaon* and *P. polydorus*. In the genus *Ephyra* (Heterocera) the dimorphic pupæ are quite uninfluenced by their surroundings, the pupal colours corresponding to those of the dimorphic larvæ.
3. The previously accepted theory, which explained the pupal colour-relation as following from the action of light upon the moist skin of the freshly-formed organism, is entirely disproved, and it is shown that the influence works upon the larva during the period which intervenes between the cessation of feeding and pupation.
4. This intervening period was carefully investigated in *V. urticae*, and it was found that, after ceasing to feed, the larvæ wander for a variable time, then rest for about 15 hours upon the surface selected for pupation, and finally hang suspended, head downwards, for about 18 hours, after which time pupation takes place. By transferring

the larvæ from one colour to another it was found that the colour influence works for about 20 hours preceding the last 12 hours of the whole period.

5. Blinding proved that the eyes do not form the organs which are influenced, and it was also shown that the complex bristles do not contain a terminal organ with this function. Experiments with conflicting colours appeared to prove that surrounding colours affect the whole surface of the larval skin, although parti-coloured pupæ were not obtained. (There is, however, some evidence for such a result in *Papilio nireus*.)

6. In all cases there are certain colours which produce no effects. In the *Vanessidæ* the brilliant metallic tints of the pupæ can be greatly influenced by the presence of gilded surfaces in the environment of the larva before pupation. This fact appears to prove that the metallic tints are essentially protective, and probably subserve concealment by their resemblance to glittering minerals, such as mica. This theory is confirmed by observations upon the habits of certain species with gilded pupæ. At the same time the gilded appearance has acquired another and opposite significance in other species, being of use in rendering the pupæ conspicuous, and thus acting as a signal of an unpleasant taste or smell.

7. The amount of pigment in the superficial layer of the cuticle in the pupæ of *Pieris brassicæ* and *P. rapæ* appears to be influenced by the spectroscopic composition of the light incident upon the larvæ before pupation.

DESCRIPTION OF PLATE 26.

Figs. 1-6, inclusive ; all $\times 2$ diameters. These figures represent a series of the pupæ of *Vanessa urticæ*, ranging from the darkest to the lightest and most golden varieties. Fig. 1, the darkest, is that represented by the degree of colour called (1) in the paper. Fig. 2, rather less dark, is called (2) in the paper. Fig. 3 represents the degree called (3). Fig. 4 represents a normal (4); while figs. 5 and 6 represent respectively the degrees of colour called normal (5) and exceptionally gilded (5).

Fig. 7. Natural size. The yellowish-green and relatively gilded form of the pupa of *Vanessa Io*, obtained by the use of yellowish-green surroundings. Similar varieties occur on the leaves of nettle.

Figs. 8 and 9. $\times 7$. These figures represent the left fore-wings and the exposed part of the hind-wings of two pupæ of *Vanessa urticæ*, showing the relative amounts of black cuticular pigment present (in the superficial layer of the cuticle) in two different varieties. Fig. 8 represents the degree of colour called (2), while fig. 9 represents that called light (3). The black parts of the networks alone represent black opaque pigment; the less dark parts of the networks are not opaque, and let the light freely through

when they are examined as transparent objects; hence the amount of opaque pigment in the lighter figure (9) is very small indeed.

Figs. 10 and 11, both $\times 7$, represent respectively the cuticle of the left pupal fore-wings of a green and a grey variety of *Vanessa Io*. There is seen to be an immense difference in the ground-colour as well as in the amount of black pigment. The dark networks seen in these and the two last figures are purely cuticular in position, and were all drawn from the empty pupa-cases from which the imagines had emerged. The dentated outline within the margin of all the fore-wings, and especially distinct in fig. 11, corresponds to the shape of the imaginal wing which was developed under this part of the pupal wing. In correspondence with this fact, the pupal pigments are altered over the area where the imaginal wing will develop. The pigmented lines indicating the nervures of the imaginal wing really stop short at the dentated margin, but they are prolonged beyond as irregular lines of pigment which produce the deceptive appearance of uninterrupted continuity. It was necessary to describe the appearance thus far, but it is a subject upon which I am now at work. The object of the figures is to give a representation of the relative amounts of cuticular pigment, and not to reproduce morphological features (viz., the venation, &c.) with exactness.

Figs. 12 and 13, both $\times 2$, represent respectively a dark and a light gilded pupa of *Vanessa atalanta*. Although the golden appearance is so much less diffused than in *V. urticae*, it is especially brilliant on the places where it does occur. These two varieties were respectively obtained by the use of dark and gilt surroundings.

Figs. 14 and 15, both natural size, represent respectively a green and a brown variety of the pupa of *Papilio machaon*. The green variety was obtained in brown surroundings, and the brown variety in green surroundings, and they are figured so as to indicate this relationship. Thus the species is obviously unaffected by the colour of its surroundings.

Figs. 16–21, inclusive, represent the colours made use of in experimenting with the larvæ of the Pieridæ. Fig. 16 = dark red; 17 = deep orange; 18 = pale yellow; 19 = green tissue-paper; 20 = pale bluish-green; 21 = dark blue.

Figs. 22 and 23. Natural size. The brown and green forms of the dimorphic pupa of *Ephyra omicronaria*. The colour of these pupæ follows that of the respective larvæ, and is unaffected by surrounding colours.

Figs. 24–30, inclusive, all $\times 2$, represent types of the different varieties of the pupæ of *Pieris brassicæ* obtained in the experiments. Fig. 24 represents the darkest variety, called (1), α , the ground-colour being of a greyish-orange; the less dark, fig. 25, represents a greyish-green, (1), β . Fig. 26

represents the less dark (1), γ , possessing a dark greyish-green ground-colour, but with an unusual absence of black patches. Fig. 27 represents a (1), β , with the normal black patches, but with the ground-colour unusually free from grey dusting, and therefore of a peculiarly bright whitish-green tint. This variety was produced in some instances by the use of a pale bluish-green background (fig. 20) and by means of a white background. Fig. 28 represents the bright yellowish-green variety called (2) in the paper, produced chiefly by the use of green tissue-paper surroundings. There is little black pigment present. Figs. 29 and 30 represent the bright-green variety called (3) in the paper, and chiefly produced by the use of orange surroundings. The latter figure is an unusually bright variety. The black pigment is even more deficient than in (2).

Fig. 31. Natural size; a green pupa of *P. rapæ*, called (5) in the paper, but with an unusual amount of pigment, so that, if it were less green, it would belong to the degree of colour called (3).

Figs. 32–41, inclusive, all $\times 2$, represent the chief varieties of the pupæ of *P. rapæ*. Fig. 32 represents the darkest variety, called (1) in the paper. Fig. 33 represents a (2), being very dark, but lighter than the last. Fig. 34 represents a light (3), with the ground-colour distinctly visible through the smaller amount of pigment. Figs. 35–38, inclusive, represent various forms of the degree of colour called (4) in the paper, 35 possessing a greyish-white ground-colour, while 36 is distinctly pinkish, 37 yellowish, and 38 greenish, and forming the transition to the greener pupæ next figured. The amount of pigment is seen to be very small in pupæ of the degrees of colour called (4) and (5). Figs. 39–41, inclusive, represent the varieties included in the degree called (5), 39 being of a pale yellowish-green, and 40 and 41 representing two aspects of the brightest green variety.

XV. *On the Homologies and Succession of the Teeth in the Dasyuridæ, with an Attempt to trace the History of the Evolution of Mammalian Teeth in general.*

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[PLATES 27, 28.]

IN the year 1867 a paper* was contributed to the Royal Society by Professor FLOWER, "On the Development and Succession of the Teeth in the Marsupialia," a paper which became at once the standard authority on the subject, and in which it was shown conclusively that among the Marsupialia only one single tooth ever had a deciduous or "milk" predecessor, and that this tooth was one homologous throughout the order, and corresponding to the last premolar of the ordinary Placental Mammals.

This paper was followed by another,† in which fresh observations were recorded on the presence or absence of a tooth-change in the Marsupials and other Mammals, and notes made on the methods of tooth-notation in use—a subject which naturally arises out of all investigations into the homologies of teeth.

Finally, in the article "Mammalia" in the new edition of the 'Encyclopædia Britannica,'‡ the same author has given a summary of our present knowledge on the subject, to which I am indebted for much information and assistance.

In the course of preparing a systematic catalogue of the Marsupials in the Natural History Museum, my progress was soon arrested by the necessity of understanding and applying the very complicated homologies of the teeth, many points on which being by no means finally settled by Professor FLOWER, and the other publications on the subject being of a very vague and conflicting nature. The group wherein the greatest difficulty occurred was the Dasyuridæ, of which only one genus, *Thylacinus*, appears to have had its dental change properly described, and among whose members I have noticed several points that I believe to be worthy of publication, and from

* 'Phil. Trans.,' 1867, p. 631.

† "Remarks on the Homologies and Notation of the Teeth of the Mammalia," 'Journ. Anat. Physiol.,' vol. 3, 1869, p. 262.

‡ Ninth edition, vol. 15, 1883, p. 349.

the study of which I have been led on to form a theory on the evolution and succession of the teeth applicable to the Mammalia in general. This theory attempts to bridge over the existing gap between the Metatheria and Eutheria, and to show how the teeth of the one may have passed into those of the other.

Before commencing, I must express my sincere thanks to Mr. R. LYDEKKER, to whose extensive knowledge of Mammalian Palæontology and its literature I am largely indebted, and with whom every point in the present paper has been fully discussed—a sifting process which has, I hope, eliminated some of the unsound conclusions to which I might have otherwise come. Throughout the course of my work he has taken considerable trouble in obtaining information on various points, and this he has at all times freely communicated to me. I feel, therefore, that he should be credited with a very large share in the results, whatever their value may be, that are put forward in the present paper. I must also record the obligations I am under to Professor FLOWER himself, whom I have consulted on several points, and who has freely given me the benefit of his large knowledge on the subject.

To confine our attention, first, to the Dasyuridæ. In this family we find the greatest amount of variation in the extent to which the change of teeth takes place, some species having a well-developed successional tooth preceded by an equally well-developed milk-molar, while, on the other hand, others have no successional tooth at all, either in the milk or permanent stage. In this family also occurs *Myrmecobius*, remarkable for being the only known heterodont Mammal normally possessing more than four true molars.

This family has also another and more vital interest for the evolutionist, arising from the presumption that it was in all probability the family in which the change from Metatherian to Eutherian occurred. This presumption is based partly on the very generalised character of the family as a whole compared to the other and more specialised groups of Marsupials, but chiefly on the strikingly exact resemblance existing between the structure of the teeth of many of its members and that found both in certain of the "Creodonta" or Carnivora Primigenia, among which it is generally supposed that the direct ancestors of the modern Carnivora should be sought for,* and also in many of the more generalised Insectivora, whose claims to the parentage of other Placentals have been advocated by Professors HUXLEY,† PARKER,‡ and others.

The family Dasyuridæ consists of the following genera, the respective numbers of their premolars and molars being placed after each :—

* See R. LYDEKKER, 'Catalogue of the Fossil Mammalia in the British Museum,' Part 5, 1887, pp. 26 (footnote) and 307.

† 'Zool. Soc. Proc.,' 1880, p. 657 and elsewhere.

‡ 'Phil. Trans.,' 1885, p. 268. 'Mammalian Descent,' 1885, p. 125 *et seq.*

| | Premolar. | Molar. |
|-------------------------------|------------------|--------|
| <i>Thylacinus</i> | 3 | 4 |
| <i>Sarcophilus</i> | 2 | 4 |
| <i>Dasyurus</i> | 2 | 4 |
| <i>Phascologale</i> | 3 | 4 |
| | (rarely 2 below) | |
| <i>Sminthopsis</i> *. | 3 | 4 |
| <i>Antechinomys</i> | 3 | 4 |
| <i>Myrmecobius</i> | 3 | 5 or 6 |

Nothing of importance seems to have been published as to the tooth-change in any of these genera, with the exception of *Thylacinus*, worked out by Professor FLOWER. This animal has its successional tooth, or "pm⁴,"† preceded by a distinct milk-molar, which is, however, never functional, and falls out exceedingly early.

In *Dasyurus* and *Sarcophilus* neither of the two premolars has a milk predecessor, and, owing to this, their homologies have not been finally determined, although Professor FLOWER has acutely suggested,‡ judging only from KREFFT's description§ of his "*Chatocercus cristicauda*," that it is the last, and usually changing, premolar which has disappeared, a suggestion which I am now in a position to prove entirely true.

On *Sminthopsis* and *Antechinomys* I propose to make no remarks, as their dentition is palpably the same as that of the common members of the genus *Phascologale*, on which my chief observations have been made, and from which I shall afterwards return to *Dasyurus* and the other members of the family.

In *Phascologale* the shape and size of the two anterior permanent premolars are always very constant, but the third and last, or pm⁴, presents us with a remarkable series of gradations in size, gradations which prove that it is undoubtedly this tooth that has altogether disappeared in *Dasyurus* and *Sarcophilus*. These gradations do not need detailed description here, especially as the figures (Plate 27, figs. 1-5) show them far more intelligibly than any description could do. It is sufficient to say that in certain species, such as *Phascologale virginie* and *penicillata* (figs. 1 and 2), the tooth is larger and longer than pm³, and that from this size a perfect set of gradations exists, down to the minute and practically functionless tooth found in *Ph. apicalis* (fig. 4), while in two species even, *Ph. cristicaudata* and *thorbeckiana*, the tooth is often altogether absent in the lower jaw.

* = *Podabrus*, GOULD, *austorum*.

† This tooth, being the homologue of the fourth premolar of other Mammals, should evidently be called by the same name, viz., pm⁴, whatever the actual number of premolars, and therefore its serial position, may be.

‡ 'Journ. Anat. Physiol.,' vol. 3, p. 277.

§ 'Zool. Soc. Proc.,' 1866, p. 485.

As to the milk dentition, those species that have a large permanent pm^4 have a distinct tricuspid milk-tooth preceding it (Plate 27, fig. 6), and persisting until a comparatively late period of life. On the other hand, when the permanent pm^4 shows a tendency to disappear, the milk-tooth would seem to be first gradually aborted; thus in *Ph. doria*, where the permanent tooth is of a medium size, the milk-tooth is quite minute and functionless, while in the still smaller-toothed *Ph. apicalis*, and also in *Ph. wallacei*, I have been unable to find any trace of a milk-tooth in the only young specimens available—the permanent tooth, however, as in the other species, still rising into its place considerably later than any of its neighbours. The actual calcification of this tooth seems also to take place much later in *Phascologale* than in any other of the tooth-changing Marsupials, so that the tooth is often not to be found beneath the bone until a very short time before its eruption.

From these observations it is clear that the normal state of a member of the present group is to have three well-developed premolars, the last one of which has a milk predecessor. Then a tooth-reduction has taken place, all of which has fallen on what is evidently a peculiarly plastic tooth, viz., pm^1 , and this, with the milk-tooth preceding it, has been decreased in various degrees, and in the end altogether suppressed, as in the allied genera *Dasyurus* (Plate 27, fig. 5) and *Sarcophilus*.

Having thus found out which of the three premolars present in *Phascologale* has disappeared in *Dasyurus* and *Sarcophilus*, we have, before we can settle the proper homologies of even these three, to discover which of the full number of four premolars, still possessed by the Eutheria, has disappeared in *Phascologale* and other Marsupials, for it has always been the natural presumption that four, and not only three, was the original typical number of premolars as much among the Marsupial as among the Placental Mammals.† Since no species now living, however, shows this number, that presumption has hitherto remained unproved, and still less has it been proved which one of the full set of four has disappeared to leave the common number of three, most authors jumping to the conclusion that, as in so many Carnivora, it is pm^1 that has been suppressed. Now, however, I am at last able to prove the first, and make out the second point to my own satisfaction, and to that of both Professor FLOWER and Mr. LYDEKKER.

When looking at a somewhat abnormal skull of *Dasyurus maculatus*, I was struck by seeing a minute projection attached to the gum, *between the two premolars*, and, being on the look-out for such a thing, I immediately suspected that it might be the

*The specimen of *Ph. penicillata* with milk dentition, from which the figure is drawn, has its third molar up and in place, and has a basal length of nearly 40 mm. as compared to about 45 mm. in fully adult specimens.

† Although some even of the highest authorities look upon three as the typical or parent number of premolars in the Marsupials; see, for example, TOMES' 'Dental Anatomy' (2nd ed.), 1882, p. 420, where, apart from this point, a most excellent account is given of the structure and development of Marsupial teeth.

missing premolar, present through atavism. I then turned to *Phascologale*, in which, as pm^4 is still retained, there seemed more hope of tracing the other missing premolar; but here, in all the commoner species, the teeth fit so closely against one another that a functionless atavistic tooth would have no chance of developing. In certain of the rarer Papuan species, however, the teeth, owing to the length of the muzzle, are more separated from one another, and, on examining all the available examples of these, I was rewarded by finding on one side of the upper jaw, in a specimen of *Ph. dorsalis* belonging to the Genoa Museum,* a large and distinct tooth (Plate 27, fig. 7) protruding from the gum between the teeth corresponding to the two anterior premolars in an ordinary *Phascologale*,† and in exactly the same position, therefore, as the minute rudiments previously found in *Dasyurus*. The tooth itself is two-rooted, precisely similar in shape to the other premolars, and is of about half the size of the first premolar in the same species. This, then, was clearly the missing premolar, and that here was its most natural place is shown by the extreme frequency with which a marked and prominent gap exists at this point in adult Marsupials, as, for example, in *Didelphys*, *Perameles*, and others.

That the original typical number of premolars in the Mammalia was four is also strikingly exemplified by several of the earlier fossil Marsupials—as, for example, by *Triconodon*, which has the full cheek-teeth formula of four premolars and four molars; by *Ctenacodon*, *Plagiulus minor*, and others; thus proving beyond question that the pm^3 discovered in the recent *Phascologale* is really an atavism and not a mere meaningless abnormality.

It results from this discovery as to the position of the missing premolar that in all the numerous Polyprotodont‡ Marsupials with three premolars these are homologous with the first, third, and fourth of the normal Mammalian dentition, and not with the second, third, and fourth, as has ordinarily been presumed to be the case.

For the abnormal specimen of *Phascologale dorsalis* we therefore obtain (on one side only) the following premolar formula:—P.M. $\frac{1.2.3.4\ddagger}{1.0.3.4}$, from which the suppression of the upper pm^3 gives us P.M. $\frac{1.0.3.4}{1.0.3.4}$, the formula in *Thylacinus*, *Phascologale*,

* For the loan of which, and of a large series of other Papuan specimens, I have to thank my friend, the Marquis G. DORIA, Director of that Museum.

† Compare the teeth of the other side in the same specimen (Plate 27, fig. 8, reversed) for the correspondence between the different premolars.

‡ As to the Diprotodonta with three premolars, although, on the one hand, in the Mesozoic Plagiulacids it was clearly pm^1 that was first lost, yet, on the other, the positions of the premolars in certain of the modern Phalangistids are such as to raise a suspicion that they also, like the Polyprotodonts, have lost pm^3 rather than pm^1 ; but in any case the loss has been independent of that in the Polyprotodonts, both groups having had four premolars some time after their separation from one another.

§ I have found this method of writing dental formulæ far superior to the ordinary one, as by it, not only the number, but the homologies of the teeth are clearly shown. Each tooth has its serial number, which is written in if the tooth is present, but is replaced by a cipher if not.

Perameles, and *Didelphys*, and from which again we obtain, by the disappearance of pm^4 , that of *Dasyurus* and *Sarcophilus*, namely P.M. $\frac{1.0.3.0}{1.0.3.0}$.

Turning then to *Myrmecobius*, with its eight or nine cheek-teeth, of which only three are commonly reckoned as premolars, I examined several young specimens in order to see if this animal, like the abnormal *Phascologale*, had not really four premolars, as no exact observations on it had been published, and the usual determination rested merely on the shape of the teeth. In fact, even if no tooth-change could be found, it seemed possible enough that the small tooth commonly reckoned as the first molar should be really a persistent milk pm^4 , the permanent tooth corresponding to it having become aborted.* This theory, however, I have been able to disprove by the examination of such a jaw as is shown in Plate 27, fig. 9. Here we find that, of the whole set of cheek-teeth (premolars and molars), the first, second, fourth, and fifth are fully up, and all much on a level with one another, while, on the other hand, the third has scarcely penetrated the gum, and stands therefore far below the level of the rest. Hence we see that this third tooth, by its very lowness of position, or rather lateness of development, is certainly the true pm^4 , even without there being any milk-tooth above it; for, had it been pm^3 , it would have been at all stages, as with other animals, fully on a level with the teeth next in front of and behind it. Of a milk-tooth preceding this pm^4 I can find no trace whatever, although it is possible that, considering the very rudimentary degree of development in which the milk-tooth occurs in the Thylacine and Koala,† it may yet be discovered in specimens younger than have yet been examined.

The correct formula of the cheek-teeth of *Myrmecobius* is thus P.M. $\frac{1.0.3.4}{1.0.3.4}$, M. $\frac{1.2.3.4.5.0}{1.2.3.4.5.6}$; and therefore, so far as regards the premolars, identical with that of *Phascologale* and *Thylacinus*.

As regards the incisors, two specimens of *Myrmecobius* in the Natural History Museum present the interesting anomaly of possessing four instead of only three lower incisors, the extra tooth being in each case clearly i^4 —a fact which proves what has generally been presumed to be the case, viz., that it is the fourth incisor that has disappeared in ordinary three-incisored Mammals.

This completes the list of the living Dasyuridæ to be referred to, but one fossil Marsupial, perhaps referable to the same family, and, although nearly the oldest-known Mammal, strongly resembling the modern *Phascologale*, has a dentition of so interesting a character as to call for special notice. This is the *Triacanthodon serrula*, described by Sir R. OWEN‡ from a single lower jaw found in the Mesozoic Purbeck

* A process which, as noted below, p. 451, has taken place in certain Placentals.

† THOMAS, 'Zool. Soc. Proc.,' 1887, p. 388.

‡ 'Mammalia of the Mesozoic Formations' (Paleontographical Society), 1870 (pub. 1871), p. 72, Pl. IV., figs. 7 and 8. Mr. LYDEKKE ('Catalogue Fossil Mammalia Brit. Mus.,' vol. 5, p. 258) considers this fossil as not generically, or even specifically, separable from *Triconodon mordax*, OWEN, described at the same time.

beds of Swanage, Dorsetshire.* An examination of this solitary lower jaw shows what is, considering the immense antiquity of the species, rather a startling fact, namely, that it had an absolutely identical tooth-change to that found in modern Marsupials. This fact is shown most clearly by the typical specimen, which happens fortunately to be in precisely the right condition to show it, namely, with the milk-premolar still in its place in the jaw, while the permanent pm⁴ is clearly visible buried in the bone beneath (see Plate 27, fig. 10). The milk-premolar is not small and nearly functionless, as it is in *Phascologale*, but is nearly as large as, and is very similar in shape to, the first molar standing just behind it. This ancient and remarkable fossil gives us, therefore, the one stage earlier than the abnormal *Phascologale* above described, having, on the presumption that its upper jaw resembled its lower, the full premolar formula of P.M. $\frac{1.2.3.4}{1.2.3.4}$, all the teeth equally well developed, and the fourth one with a large and functional milk predecessor.†

We may now consider this history of the evolution of the premolars of the Dasyuridæ as fairly proved, and may represent it diagrammatically as follows:—

| | Fig. 1. P. M. | | | | Process. | Examples. |
|------|------------------|---|---|--------|--|--|
| | 1 | 2 | 3 | 4 | | |
| I | ✓ | ✓ | ✓ | ✓ U | Complete set | <i>Triacanthodon</i> . |
| II. | ✓ | ✓ | ✓ | ✓ U | Reduction in size of pm ³ . . . | Abnormal <i>Phascologale</i> . |
| III. | ✓ | ○ | ✓ | ✓ U | Loss of pm ³ | Ordinary <i>Phascologale</i> . |
| IV. | ✓ | ○ | ✓ | ✓ | Reduction in size of pm ⁴ . . . | <i>Phascologale apicalis</i> , &c. |
| V. | ✓ | ○ | ✓ | ○ | Loss of pm ⁴ | <i>Dasyurus</i> and <i>Sarcophilus</i> . |

In this diagram the permanent teeth are represented under their respective serial numbers by a ✓ if present, and by an ○ if absent, while the milk-tooth is similarly shown by a shaded U if present, but is altogether unrepresented if absent.

We will now pass from these details of individual species to the larger question as to the steps by which the primitive ancestral set of Mammalian teeth has become

* This specimen is also referred to by Professor Flower in an address to the Odontological Society ('Odontol. Soc. Trans.,' vol 3, 1871, p. 220), and he there, on the then unverified assumption that it possessed a changing pm⁴, made certain remarks on the probable direction of the evolution of a Diphodont dentition—remarks which all the evidence at my disposal most fully bears out.

† As Professor Flower has pointed out in his paper just quoted, the type-specimen of *Triconodon occisor*, Owen (figured *op. cit.*, Pl. IV., fig. 2), found in the same beds, also shows traces of having had a changing pm⁴, the latter tooth being very markedly retarded in development, as if a milk predecessor had only just been lost from above it. There seems, in fact, to be every reason to suppose that all the Purbeck Polyprotodonts had a similar tooth-change, judging from such indications as may be gathered from the relative positions of the teeth.

modified into the very various forms of modern dentition, and especially as to the passage from the Marsupial to the Placental style of dentition.

The first and most fundamental question that arises is this :—Is the rudimentary tooth-change now found in the Marsupials the last remnant of a complete change present both in their ancestors and in those of the Placentalia, or does it represent an early stage in the first formation of such a complete change, the Marsupials being still in a backward condition, out of which the Eutheria have long ago passed ?

To my mind it is perfectly clear that it is the second and not the first question that should be answered in the affirmative ; although, so far as I can find, all the Continental and many of the English naturalists think the opposite—a view, however, that, although easy and obvious at first sight, I cannot for one moment believe to be correct. When we consider that in every character of their organisation the Marsupials are infinitely behind and at a lower stage of evolution than the Placental Mammals, it would appear to be a total subversion of all the ordinary rules to suppose that in this one character of their dentition they should have passed on in advance of all the other Mammals, and, having gone through the condition in which the latter now are, should have again nearly evolved away that process of tooth-change which is to its Placental possessors so evidently advantageous. It would be to my mind inconceivable that this should be the case, considering how universal among the Eutheria a more or less complete tooth-change is, and how useful it has proved to be to them, as evidenced by the very fact of their so wholly supplanting the more lowly organised Marsupials—more lowly organised in their dental as well as in their other characters, and not further advanced, as would have to be presumed were their teeth looked upon as a later development of a fully Diphyodont set.

And again, the mere fact that five out of the six families of Marsupials, natives both of Australia and America, have, with the comparatively unimportant exceptions already noted as occurring among the Dasyuridæ, arrived at precisely the same stage of tooth-change is itself a very strong argument in favour of the view now advocated ; for, were the modern tooth-change a remnant of a fuller one, we should naturally expect that, under the very various conditions of the struggle for existence, equally various degrees of reduction would have been attained to. On the other hand, we should be most unlikely to find, as is now the case, more than 90 per cent. of the existing and fossil Marsupials changing one single tooth, and one only, and the small remainder merely differing from them in a direction away from and not towards that fuller tooth-change of which it is said their ancestors were once possessed.

Of the one form of evidence needed to give any weight to the opposite theory, namely, that derived from Palæontology, there seems not to be one atom, no fossil Marsupials having ever been found showing traces of a larger amount of tooth-change than the recent ones, nor do I think such a discovery likely to be made, as, to the best of my belief, no such animal has ever existed. This view is very much strengthened by the fact that, as already noted, one of the oldest Mammals known,

the Mesozoic *Triacanthodon*, had precisely the same, and no more, tooth-change than the modern Marsupials.

For these various reasons, therefore, we may, I think, take it for granted that the ancestors of the Marsupials never had at any time a more complete change of dentition than they have now, and that they arrived at their present state at an immensely early period, since which time they have as a whole practically stood still, except that a few isolated forms have, comparatively recently, lost again even the small amount of change they once possessed.

The second question, and one equally vital, is as to whether the milk set of teeth is the original primary set with the permanent one superadded to it, as believed by many naturalists, and especially by embryologists, or *vice versa*: a question with which Professor FLOWER has dealt in the first of his papers above referred to.* His conclusion was that the permanent dentition was the original one, and that the milk set had been afterwards developed as an addition to it—a view to which, although inclined at first to disagree, I have now become a firm adherent. To this opinion I have come by finding the impossibility of working out the general homologies of the teeth on the basis of the opposite view, and by the comparison of an infinitely larger number of specimens of various sorts than even Professor FLOWER had access to. The chief cause of the prevalence of the opinion that the permanent dentition is a later development than the milk is the deceptive appearance presented in the early stages of tooth-development, when the germ of the permanent tooth is first seen as a bud growing out from that of the milk-tooth, whence it has naturally been supposed that the latter was the primary and the former the secondary development. Even should this appearance of budding off, however, be entirely correct—and the fact itself is strongly denied by R. BAUMÉ†—it may be argued that, considering the uniform direction of the evidence drawn from later stages, there is no sufficient reason to deny the possibility of a secondary organ, whose very *raison d'être*, as in the case of the milk-tooth, is its speedy and precocious development, so overshadowing in size and rapidity of growth what is really the primary as to make the latter appear as its bud, and therefore, although falsely, as a secondary and subsidiary growth.

A second, apparently adverse, argument may be drawn from the few instances known of milk-teeth‡ developed, and remaining through life, without having, or only rarely having, true permanent successors, as in the anterior premolars of the Proboscidea§ and some of the Perissodactyla,|| but these are clearly due to the teeth

* 'Phil. Trans.,' 1867, p. 639.

† 'Odontologische Forschungen : Versuch einer Entwicklungsgeschichte des Gebisses,' 1882, p. 75.

‡ *I.e.*, the homologues of the corresponding milk-, and not permanent, teeth in the allied species.

§ R. LYDEKKER, 'Cat. Foss. Mamm. Brit. Mus.,' Part 4, 1886. (*Introduction*, p. vii.)

|| R. LYDEKKER, 'Bengal, Asiat. Soc. Journ.,' vol. 49, 1880, p. 135. This interpretation by Mr. LYDEKKER of the homologies of the non-changing pm¹ in the Rhinoceroses has been called in question, but his evidence, drawn from an abnormal Rhinoceros skull in the Calcutta Museum, seems to me to be fully sufficient to support the conclusions he came to—conclusions with which all the specimens that I have seen quite agree.

having passed through a complete cycle of evolution, the "permanent" tooth first developing, then having a milk-tooth superadded to it, and finally aborting itself and leaving its milk representative still persistent.

These two questions answered, we come to the consideration of the phenomena observable during the growth of a milk-tooth above its permanent successor; not the early and embryological ones, into which I do not propose to enter, but such as may be studied on the rich material of skulls of different ages available to me in the collection of the Natural History Museum.

Taking now the skull of a tooth-changing Marsupial in which the first teeth are just appearing, we see that the single milk-premolar* comes up at about the same time as the true permanent premolars anterior to it; that the first molar quickly succeeds and the other molars follow, but that almost invariably, whether there is a change or not, the true pm^4 is considerably later in its development than the other teeth, and, especially, than either pm^3 or m^1 . In the original production, therefore, of a milk-tooth above one of the other teeth, say pm^3 , whose summit is, to commence with, fully equal in height at all stages of development to the summit of the milk-tooth of pm^4 standing just behind it, we see that a change of position is necessary in this pm^3 before a milk-tooth can be developed over it, a change which can apparently only be brought about by the retardation of its growth, and its approximation thereby to the state in which pm^4 now is.

Should this supposition be true, we should expect to find that, anterior to the first appearance of a milk-tooth in any group, specimens would be found showing a preliminary retardation of the individual teeth over which, in a later generation, milk-teeth were to be developed. The difficulties in the way of understanding how the ordinary processes of evolution can have first brought about such a preliminary retardation are, of course, considerable, unless it be that the retardation is itself a favourable character, by its preventing the undue crowding of the young animal's mouth, while, at the same time, the full number of teeth are developed for use by the adult. In this case it would be comparatively easy to understand the assumption of a milk dentition by the two steps, each favourable in itself—(1) of a retardation for packing purposes of the permanent tooth in some large-toothed form, followed, in a later generation, by (2) the temporary development of a deciduous tooth in one of its descendants with the teeth so small that the gap in the tooth-row during youth, inherited from large-toothed ancestors, had become a defect to be remedied in this most effective manner.

Turning now to the actual facts, we find that there is among the Marsupials a

* It seems better to use this term rather than "milk-molar," as the milk-teeth have nothing to do with the true molars, and that name is, therefore, productive of constant confusion. And, again, the use of the word "deciduous" instead of "milk" seems to be inadvisable, as both the molars and premolars of the second set are often themselves deciduous, while occasionally those of the first or milk set remain throughout life.

hitherto unnoticed, but most striking and peculiar, retardation in the development of the first upper incisors in all the three Polyprotodont families, these families being the very ones in which, as the incisors are not so specialised in another direction as are those of the Diprotodonts, we should most expect to find traces of the development of milk incisors. In half-grown specimens, such as those of *Sarcophilus ursinus* and *Phascologale wallacei* (figured Plate 27, figs. 11 and 12), at a time when the three outer incisors are fully up and in use, the first pair, the largest in the adult (*Ph. wallacei*, fig. 13), are still quite minute, with their points only just projecting above the bone, and altogether in a very marked condition of retardation. And the same appears to be the case in young specimens of Didelphyidæ, Peramelidæ, and other Dasyuridæ, in many of which the first incisors in the fully adult animal are by far the largest of all. The theory now suggested, therefore, about these Marsupials with retarded first incisors, is that they represent at the present day the stage at about which the common ancestors of the Metatheria and Eutheria diverged from each other—a stage when the teeth were, as it were, preparing themselves for the assumption of milk predecessors, a process which has in the latter group been continued onwards until the complete Diphyodont condition has been attained. On the other hand, in the former the process has, except in the case of pm^4 , never gone beyond the initial stage of the retardation of i^1 , a stage which has itself been continued owing to its own inherent value.

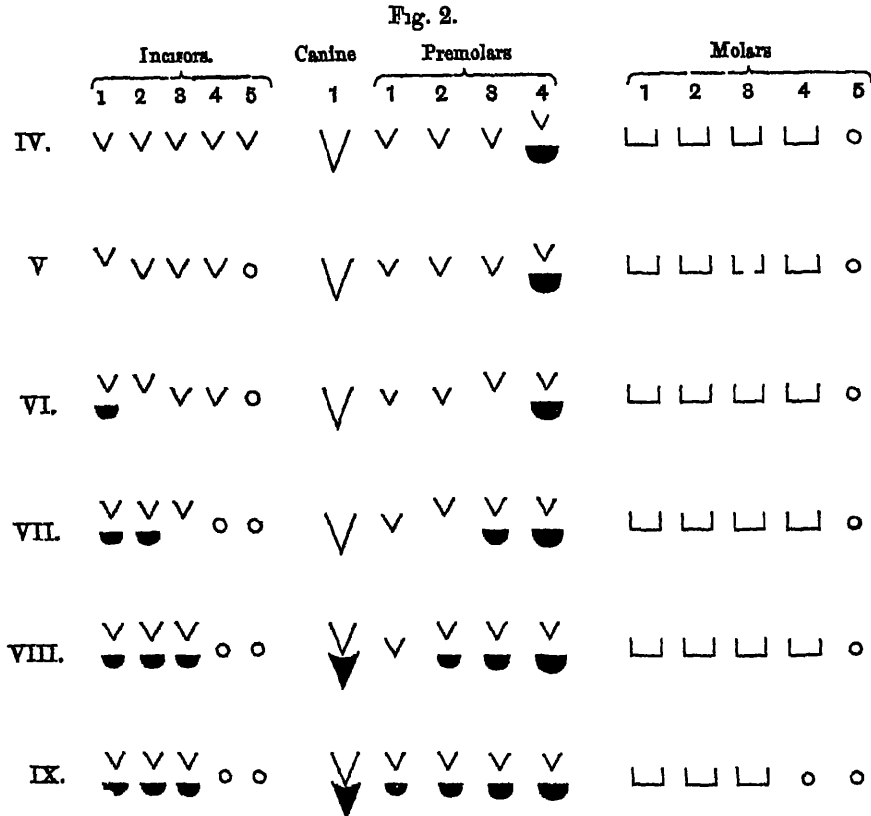
For this view as to the first incisors it may also be urged that the most likely of all the teeth to undergo a marked alteration of any sort would be those at the extreme ends of the series, judging by the manner in which, throughout the Mammalia, the first incisor and the last molar show themselves plastic in readily taking on characters not, or only much later, found in the neighbouring teeth.

Before leaving this subject, I would wish to point out that it is by no means essential to the general views here advocated that this suggestion as to the first incisors should be correct, but only that, as some tooth or other must have been the first to follow the example of pm^4 in developing a milk predecessor, the first incisor, even apart from its retardation, is at least as likely as any other to have been that tooth, while on this theory we also gain a possible explanation of the same very curious retardation. Should, however, future palæontological research show that any other tooth, say the canine or pm^3 , took on a milk predecessor before i^1 , it would only disprove the present suggestion without in any way invalidating the general conclusions come to.

In order now to put into order the various suggestions above made, and to utilise them for the purpose of making out the past history of tooth evolution, we will commence by drawing up diagrams on the same principle as in fig. 1, but the whole set of teeth, instead of only the premolars, is taken into account (fig. 2).^{*} Here IV.

^{*} In these diagrams and their explanations the teeth of the upper jaw only are referred to, as it is there alone that, owing to the presence of the premaxillo-maxillary suture, the true relations of the

represents a generalised Marsupial dentition with five incisors,* four molars, and four premolars, of which the last is retarded, and has a milk-tooth superadded to it. The next advance on this in the Placental direction would be V., obtained by the retardation of i^1 and the suppression of i^5 , a stage exactly represented in the abnormal *Phas-*



cologale above described. Then, if the above theory is correct, should follow, first, VI,† where the retarded i^1 has developed a milk predecessor, and i^3 and pm^3 are retarded; secondly, VII,‡ in which the development of milk-teeth has extended from in front backwards and from behind forwards, and i^4 has dropped out; and, thirdly, VIII, which would be just such a generalised Placental dentition as is now possessed

teeth can be made out with certainty. But whatever changes the upper teeth have passed through must of necessity have also been undergone, *pari passu*, by the lower.

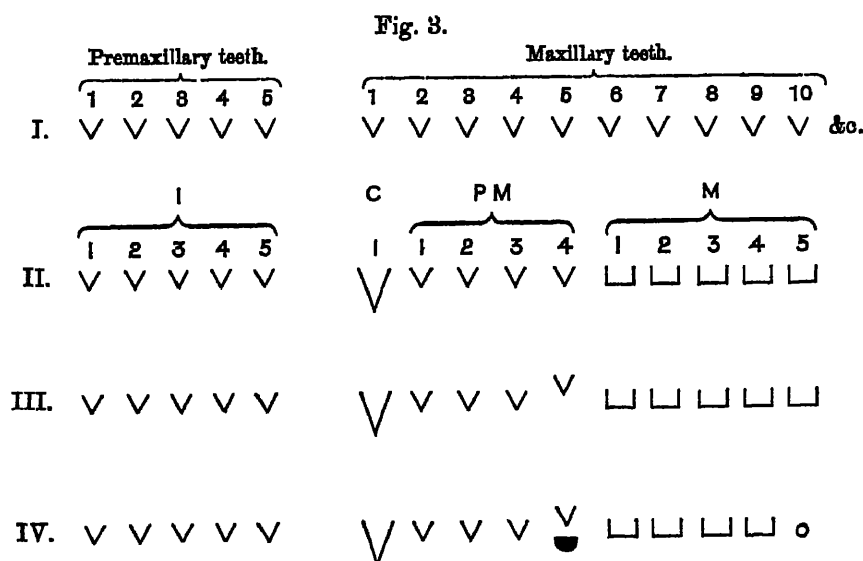
* As in *Didelphys* and some *Perameles*.

† Some of the ramifications of these intermediate stages may be represented by certain of the Eocene Creodonts of North America, as, for example, by *Trisodon quivirensis*, CORN, 'American Naturalist,' vol. 15, 1881, p. 667 (figured *op. cit.*, vol. 18, 1884, p. 257), which is said to have changed its two posterior premolars only, but the published descriptions of this interesting fossil and its allies are so incomplete and confusing that it is difficult to obtain any exact idea of their dental characters. It must also be noted that, although Professor CORN looks upon and argues from *Trisodon* as an animal only changing its pm^3 and pm^4 , there appears to be no evidence that it did not also change its pm^2 , canine and incisors, like *Hyænodon* and the Carnivora of the present day.

by *Otocyon*,* viz., three incisors and one canine, all changing; four premolars, of which the last three change; and four molars. Finally, IX. is the still further development shown in the Tapir, Hyrax, and one or two other forms, in which pm^1 also changes, and, as in the vast majority of Placental Mammals, m^4 is lost.

If, now, we attempt in the same manner to trace the history of the tooth-changes upwards to the earlier forms from the Marsupial dentition, instead of downwards to the later ones, we obtain a diagram as follows:—

Here IV. is, as before, the generalised Marsupial dentition, as already described III. would then be the stage preceding it, where one or more additional posterior molars are still habitually retained, and where the milk predecessor to pm^4 has not yet been developed, although that tooth is in the preliminary stage of retardation. In II. we get back to a condition in which the teeth are about equal to one another in



their rate of development, none being retarded, and only show a commencement or first sketching out of the division into canines,† premolars, and molars by a lengthening of the anterior and a broadening of several of the posterior maxillary teeth. Finally, in I. the teeth would have been of a purely homodont character, only divisible into five premaxillary and a variable number of maxillary teeth. Of these maxillary teeth, it would seem to have been generally the fifth ($= pm^4$) which first developed a milk predecessor, and thereby became, with the three non-caniniform teeth in front of it, a “premolar.” Where the maxillary teeth exceeded nine, the increase

* Whether this animal has returned to, or retained, its ancestral number of molars is still doubtful, but it presents, in any case, an interesting example of a stage of dentition through which the line of Placental Mammals must have passed. (Of. HUXLEY, ‘Zool. Soc. Proc.’, 1880, p. 256 *et seq.*)

† Although, as urged by MOSELEY and LANKESTER (‘Journ. Anat. Physiol.’ vol. 3, 1869, p. 73), the canine is not essentially a distinct tooth from the premolars, yet it was evidently very early specialised, as is shown by such forms as the Mesozoic *Stylodon pusillus*, OWEN.

appears to have been wholly in the number of the true molars, and not of the premolars, as is particularly well illustrated by the early representatives of the many-toothed Odontocete Cetacea,* and also by the great majority of the Mesozoic Mammalia, many of which,† while still having only four premolars, have from five to eight true molars. In fact, the earliest forms seem to have normally had a considerable number of molars, but these were soon reduced down to four in the Metatheria, and later to three or less in the Eutheria, leaving a few isolated forms,‡ with larger numbers as remnants, retaining what the common ancestors of all had once possessed. Two only of the many described genera of these ancient forms have been said to have more than four premolars, namely, *Amphitherium* and *Amphilestes*, each described as having six premolars and six molars §; but, as even this determination is disputed,|| and in any case rests entirely on the form of the teeth, and not on a knowledge of their manner of changing, it can hardly be said to prove incorrect the practically universal rule that the typical number of premolars is, and has always been, at least since middle Mesozoic times, four, and four only.¶

Whether the teeth in the first stage of all were rooted or rootless is very doubtful, but the probability seems to be on the whole that they were simple conical teeth, rootless for part, if not the whole, of the animal's life, and possibly not unlike those now possessed by some of the Dasypodidæ.**

This first stage in the Mammalian dental series would probably very fairly represent, so far as can be judged, the dentition of the Prototheria, the toothed and generalised ancestors of the living, and now highly specialised, Monotremata, which there is every presumption for believing, as Professor FLOWER has pointed out,†† were for some time both Homodont and Monophyodont.

If we now combine these diagrams and tabulate the various processes and examples already described, we obtain (Plate 28, I. to IX.) a complete diagram of the tooth

* E.g., *Squalodon*, which has the following formula:— $I. \frac{1}{2} C. \frac{1}{2} P.M. \frac{1}{2} M. 7 \times 2 = 60$. FLOWER, 'Encycl. Brit.' 9th edit., Article "Mammalia."

† E.g., *Achyrodon manus*, *Stylodon pusillus*, &c. See OWEN, 'Mesozoic Mammalia' (Palæontogr. Society, 1870), 1871. Plate 2, figs. 6, 14, and 17.

‡ E.g., *Myrmecobius*, *Bettongia*, which has not infrequently five true molars, and the many-toothed Edentates.

§ OWEN, *loc. cit.*, Plate 1, figs. 21–23.

|| LYNCKEY, 'Cat. Foss. Mamm. Brit. Mus.', Part 5, 1887, p. 271, footnote.

¶ A single instance is, however, known to me of a true Heterodont and Diphyodont Mammal with five premolars, namely, *Rhinogale melleri*, GRAY, a member of the Herpestine section of the Viverridæ. Of this remarkable species, however, only one skull is as yet known (figured 'Zool. Soc. Proc.', 1864, p. 574), so that no positive deductions can be drawn from it. It may be either that the supernumerary premolar is a mere accidental duplication of one of the other premolars, or that one of the milk-premolars has been retained in position, but these points can only be settled by the examination of further specimens of the species.

** See BAUM, *op. cit.*, p. 152, &c.

†† 'Odontol. Soc. Trans.', vol. 3, 1871, p. 221.

changes that have taken place from the earliest Prototherian form (I.) down to such a fully developed Diphyodont dentition as is now possessed by *Tapirus* (IX.), from which again, or more directly from VIII., there have arisen, by the different processes of tooth variation, all the numerous forms of modern Eutherian dentition as exemplified on the diagram by X. (*Elephas*),* XI. (*Hydromys*), XII. (*Felis*), or XIII. (*Chiromys*).†

Again, working in another direction, we can obtain an idea of the tooth evolution that has taken place in the Marsupials by starting from the "generalised Marsupial"

Fig. 4.

| Stage. | Dentition. | | | | | | | | | | Process. | Remarks or examples. | | | | |
|--------|------------|---|---|---|---|---|----|---|---|---|----------|----------------------|---|---|---|---|
| | I | | | | | C | PM | | | | M | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 1 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 5 | |
| IV. | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | □ | □ | □ | □ | ○ | Generalised Marsupial. |
| IVa. | √ | √ | √ | √ | √ | √ | √ | ○ | √ | √ | □ | □ | □ | □ | ○ | Loss of pm ³ . <i>Didelphys</i> and most <i>Perameles</i> |
| IVb. | √ | √ | √ | √ | ○ | √ | √ | ○ | √ | √ | □ | □ | □ | □ | ○ | Loss of i ¹ . <i>Phascologale</i> , <i>Thylacynus</i> , and some <i>Perameles</i> . |
| IVc. | √ | √ | √ | √ | ○ | √ | √ | ○ | √ | ○ | □ | □ | □ | □ | □ | Loss of milk pm ⁴ and retention of m ¹ . <i>Myrmecobius</i> . |
| IVd. | √ | √ | √ | √ | ○ | √ | √ | ○ | √ | ○ | □ | □ | □ | □ | ○ | Loss of pm ⁴ . <i>Dasyurus</i> |
| IVe. | √ | √ | √ | ○ | ○ | √ | √ | ○ | √ | √ | □ | □ | □ | □ | ○ | Loss of i ⁴ from IVb. <i>Pseudochirus</i> , <i>Thylacoleo</i> , &c. |
| IVf. | √ | √ | √ | ○ | ○ | √ | ○ | ○ | ○ | √ | □ | □ | □ | □ | ○ | Loss of pms ¹ and ² . Reduction in size of milk pm ⁴ . <i>Phascolarctos</i> . |
| IVg. | √ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | √ | □ | □ | □ | □ | ○ | Loss of i ² and ³ . canine and milk pm ⁴ , from IVf. <i>Phascologale</i> . |
| IVh. | √ | √ | √ | ○ | ○ | ○ | ○ | ○ | √ | √ | □ | □ | □ | □ | ○ | Loss of canine and pm ¹ from IVd. <i>Macropus</i> † |

stage represented by IV. in figs. 1 and 2, at which point the direct evolution of Diphyodontism was in their case arrested, and drawing up a similar diagram (fig. 4) to those already given. This diagram, however, is quite simple, and only depends on the loss or variation of individual teeth, and therefore does not need any detailed explanation beyond what is placed under the headings of "Process" and "Remarks" in the diagram itself. The position of this line of dental evolution in the general system is shown on Plate 2 under the heading of "Metatherian branch."

* No evidence as yet exists as to which of the three incisors is represented by the Elephant's tusk, which is here only provisionally called i¹.

† See PETERS, 'Berlin, Akad. Abhandl.,' 1865 (*Phys.*), p. 79 *et seq.*, Pl. 2.

‡ Of course it is not pretended that the dentitions of these animals are directly descended from one another, but the diagram serves to show by what steps any individual dentition may have been evolved from the generalised type.

Of the other Mammalian orders all fall easily enough into their places in this scheme,* with two exceptions, namely the Cetacea and Edentata. As regards the first, the general drift of the evidence seems to be that their ancestors have passed through a stage with a more or less complete milk dentition, which has gradually been again aborted,† its place being taken in the Odontocetes by the large and quasi-vegetative increase in the number of the molars, and in the Mystacocetes by the baleen, which latter has so fulfilled all the requirements of the animal that the "permanent" or original dentition has also been reduced to the position of a useless atavism, shed or absorbed before birth, and not playing any functional part in the life of the animal.

In the Edentata, on the other hand, we find, as is well known, characteristics wholly at variance with those of all other Mammals. In fact, a study of the teeth of this

| Stage. | Dentition. | | Process. | Remarks and examples. |
|--------|--------------------|----------------------------------|-------------------------------|---|
| | Premaxillary teeth | Maxillary teeth. | | |
| | 1 2 3 4 5 | 1 2 3 4 5 6 7 8 9 | | |
| a | √ √ √ √ √ | √ √ √ √ √ √ √ √ √ | | Generalised Mammal (Stage I. of fig. 3). |
| β | o o o o √ | √ √ √ √ √ √ √ √ o | Loss of i ¹⁻⁴ . | <i>Dasypus</i> . |
| γ | o o o o o | √ √ √ √ √ √ √ √ (√) | Loss of i ⁵ . | <i>Xenurus</i> , &c |
| δ | o o o o o | √ √ √ √ √ √ √ √ √, &c., up to 25 | Increase of number of molars. | <i>Priodon</i> . |
| ε | o o o o o | √ √ √ √ √ o o o o | Decrease of number of molars. | <i>Bradypodidæ</i> , <i>Megatheriidæ</i> . |
| ζ | o o o o o | o o o o o o o o o | Total loss of teeth. | <i>Myrmecophagidæ</i> , <i>Manidæ</i> . |
| η | o o o o o | √ √ √ √ √ √ √ √ o | Assumption of milk-teeth. | <i>Tatusia</i> . |

order soon induces a belief that the variance is so great as to preclude the possibility of the Edentates lying within the same lines of development as other Mammals, a belief that tallies exactly with the conclusions of Professor PARKER,‡ drawn from the embryology of the group.

With this idea we may look upon the dentition of the Edentates as also based, like that of other Mammals, on Stage I. of the diagrams (fig. 3, and Plate 28), but modified in a different direction, and one peculiar to itself. Working out this suggestion, as before, by means of diagrams, we have the same Stage I. (fig. 5, a), in which the teeth are simple, and only divisible into five premaxillary and a variable number of maxillary

* Except that, of course, innumerable problems still remain to be settled as to which of the typical number of teeth are present or have disappeared in the different groups. This is, however, merely a matter of detail, and does not affect the scheme in general.

† Cf. FLOWER, 'Journ. Anat. Physiol.,' vol. 3, 1869, p. 271.

‡ 'Phil. Trans.,' 1885, p. 116. 'Mammalian Descent,' p. 97, 1885.

teeth. Then, with merely the slight modification of the loss of the first four premaxillary teeth, we get β , the dentition of *Dasypus*; next, by the suppression of the last premaxillary tooth as well, we obtain γ , that of *Xenurus*, *Tolypeutes*, and *Chlamydomorphus*. Then, on the one hand, a simple increase* of the number of the maxillary teeth to 20 or more results in δ , the dentition of *Priodon*, and, on the other, a similar decrease to 5 in that of *Bradypus* and its allies, both recent and fossil (ϵ). This reduction is then carried out to its extreme in *Myrmecophaga* and *Manis* (ζ), both entirely toothless. Finally, η based on γ , with the superaddition of a nearly complete set of milk-teeth, gives us the remarkable and unique double dentition of the genus *Tatusia*.

Should this view of the derivation of the Edentata be correct, it is evident that their line of development should have a name corresponding to the useful evolutionary terms suggested by Professor HUXLEY† for the great Mammalian groups, and since almost universally used. I would, therefore, altogether remove the Edentates from the "Eutheria" and call them the "Paratheria," to indicate their position by the side of, but separate from, the other Mammals.

One genus of Edentates has not been mentioned, namely *Orycteropus*, with its extraordinary canaliculated compound teeth, wholly unique among Mammals, and only comparable to those of certain Fish. I can, however, at present make no suggestion as to the origin or evolution of these teeth, there being as yet no evidence bearing upon them in any way.

Putting now together all the diagrams above worked out, we obtain the general genealogical Table (Plate 28), in which the three great lines of development are shown, viz., the main Proto-meta-eutherian stem, I. to XIII., at the bottom of which all the modern Placentals stand; I.-a to I.- η , the Edentate or Paratherian line; and I. to IV., and from IV. to IV.-h, that of the Marsupials.

The influence that these theories, if correct, will exercise on tooth-notation is a matter of detail which will require proper working out in each group; but it is evident that such generalisations as that missing incisors are always gone from the posterior and missing premolars from the anterior ends of the series are quite untenable, and that every group must have the homologies of its teeth worked out for itself, and not merely put down under the influence of any such general rule. This influence has even caused such eccentricities as the numbering of the premolars from behind forwards, a proceeding which would, for example, result in the two premolars of *Dasypus* being called (from before backwards) pm^2 and pm^1 , instead of, as they have above been shown to be, pm^1 and pm^2 respectively.

Having now put forward the views gained by my own examination of specimens, there remains to be noticed the other published work on the subject. The numerous contributions to the history of teeth made by Sir RICHARD OWEN during the last 50

* Or, perhaps, rather a retention of the numerous teeth no doubt possessed by the earliest Prototheria.

† 'Zool. Soc. Proc.,' 1880, p. 653 *et seq.*

years are so well known as to need no more than a passing mention from me. In all questions of fact, especially in connection with the fossil forms, I have gained great assistance from them, an assistance for which I must make my due acknowledgment. Of Professor FLOWER's papers, already referred to, it need only be here remarked that every opinion he expressed has been fully confirmed, and that any advance on his papers is due to the examination of a far larger series of specimens than were available to him—an examination carried out very largely on the lines indicated by him. To Mr. C. S. TOMES's invaluable work, 'Dental Anatomy,' I am also largely indebted for information in regard to the growth and early development of the teeth.

Of the more recent foreign contributions to the subject, the most important are those of Professor E. D. COPE* and of Dr. R. BAUME†; but the differences between their views and those now brought forward are so considerable, and involve so much detailed argument, that a criticism upon them would here be out of place. It must, therefore, suffice to say that their respective views on the descent and homologies of teeth have been fully taken into consideration during the preparation of the present paper.

In conclusion, as it is to the general advantage that true theories should be confirmed and untrue ones soon exploded, I have thought it useful to draw up a few notes on the possible or probable discoveries relating to this subject, in order that they may be looked for and their true bearing understood by persons interested in, and having opportunities for making observations on, tooth-homologies.

1. The discovery in a recent Marsupial of a milk-tooth preceding one of the permanent set other than pm^4 , and especially i^1 . This discovery, although unlikely to be made, would on the whole be confirmatory of the views above advocated, as it would show that the process of the formation of milk-teeth is still going on in the Marsupials on the lines believed to have been followed by the common ancestors both of them and of the Eutheria.

2. The same in a fossil, undoubtedly Marsupial, and in its other characters allied to, and perhaps ancestral to, the living forms. This would be obviously entirely fatal, as it would show that the view as to the Marsupial tooth-change being a remnant and not a commencement of a full change is, after all, the true one. On the other hand, it is just possible that some of the extinct Marsupials may have antedated the existing species in the formation of a fuller milk dentition, and have then died out from some unexplained cause. A full, and not a rudimentary, tooth-change in a fossil Marsupial would, therefore, be the best and most final disproof of my views. That such a discovery, however, will ever be made, I cannot believe, especially considering the astonishing persistence of precisely the same amount of tooth-change from the Mesozoic to the modern Marsupials.

* Papers in the 'American Naturalist,' 'Proceedings of the American Philosophical Society,' and elsewhere.

† 'Odontologische Forschungen.—Versuch einer Entwicklungsgeschichte des Gebisses,' 1882.

3. Fossil Eutherian Mammals, showing an intermediate degree of tooth-change between the modern Eutheria and Metatheria, would obviously be highly favourable to the views now put forward as furnishing some of the intermediate stages corresponding to those above called Stages VI. and VII.

4. The discovery of a rudimentary *successor* to any of the premolars or other teeth now unchanging in the Marsupials. This also would be fatal, as it would show that it is, after all, the permanent and not the milk set of teeth which is superadded. It is, however, possible that at the first commencement of the assumption of a fuller change by the Marsupial ancestors of the Placental Mammals the Marsupial characters of inflected jaw, imperfect palate, &c., were retained for some time after the rudimentary tooth-change had been improved upon. In some Mesozoic fossils, therefore, the Metatherian osteological and Eutherian dental characters may be found to have co-existed for a certain time without invalidating the views above expressed.

5. Fossil Edentates with more premaxillary teeth than one, or, in other groups than true *Dasypus*, with any at all, would be confirmatory of the suggestion that the Edentates descended from the same Stage I. as other Mammals, and had lost four, or all, of their original number of five premaxillary teeth.

6. Further instances of the atavistic recurrence of usually absent teeth in all groups of Mammals are much needed for the working out of the tooth-homologies in the different groups.

On any of these points it will be most important to have information, and I hope that such, whether favourable or adverse to my views, will, if correct and properly authenticated, be soon forthcoming.

EXPLANATION OF THE PLATES.

PLATE 27.

Fig. 1. Anterior cheek-teeth of *Phascologale virginia*.

Fig. 2. „ „ „ „ *penicillata*.

Fig. 3. „ „ „ „ *thorbeckiana*.

Fig. 4. „ „ „ „ *apicalis*.

Fig. 5. „ „ „ „ *Dasyurus viverrinus*.

This series shows the gradual decrease in size, and ultimate loss, of the last premolar (pm⁴).

Fig. 6. Anterior cheek-teeth of *Phascologale penicillata*, showing the milk pm⁴ in position, and its successor above it still buried in the bone.

Fig. 7. Anterior cheek-teeth, left side, of abnormal specimen of *Phascologale dorsalis*, showing atavistic second premolar (pm³) in position.

- Fig. 8. Reversed drawing of the right side of the same specimen, to show the relative positions of the teeth.
- Fig. 9. Lower jaw of *Myrmecobius*, showing retarded eruption of pm^4 .
- Fig. 10. Lower jaw of *Triacanthodon serrula*, showing the germ of the permanent pm^4 buried in the bone (after OWEN).
- Fig. 11. Front of upper jaw of a young *Sarcophilus ursinus*, showing retarded development of the first incisor.
- Fig. 12. The same in *Phascologale wallacei*.
- Fig. 13. Adult *Phascologale wallacei*, showing relative size of first incisor when fully developed.

PLATE 28.

Diagrammatic representation of Mammalian tooth-evolution. On the right is the main stem of evolution, from the Prototherian to the Eutherian dentition. On the left, above, is the Paratherian (Edentate), and below, the continued Metatherian branch.

- Main Stem.—
- I. Generalised Prototherian dentition.
 - II. and III. Intermediate stages towards—
 - IV. Generalised Metatherian dentition.
 - V., VI., and VII. Intermediate stages towards—
 - VIII. and IX. Generalised Eutherian dentitions.
 - X. to XIII. Examples of specialised Eutherian dentitions: X., *Elephas*; XI., *Hydromys*; XII., *Felis*; XIII., *Chiromys*.
- Paratherian Stem.—Starting from I. or α , the generalised Prototherian dentition.
- β , *Dasypus*; ϵ , Bradypodidæ, Megatheriidæ; ζ , Manidæ and Myrmecophagidæ; η , *Tatusia*.
- Metatherian Branch.—Starting from I., the Prototherian, to IV., the generalised Metatherian dentition.
- IV. a. *Didelphys*, and most *Perameles*.
 - IV. b. *Phascologale*, *Thylacinus*, and some *Perameles*.
 - IV. c. *Myrmecobius*.
 - IV. d. *Dasyurus*.

XVI. *The Embryology of Monotremata and Marsupialia.*—PART I.

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[PLATES 29–31.]

INTRODUCTION.

IN 1882 the late Professor F. M. BALFOUR suggested my undertaking the study of the development of the peculiar Australian Mammalia and *Ceratodus*. In 1883 I decided to carry out this suggestion, and was elected to the travelling studentship founded in BALFOUR's memory.

The Committee of the Royal Society appointed to administer the Government Grant for the endowment of research gave me a sum of £400 for equipment.

A Sub-committee handed on to me the sum of £100, which they had for a similar object obtained from the same fund.

Through the courtesy of the President of the Royal Society, I obtained letters of introduction from the Colonial Office to the Governors of New South Wales, Victoria, and Queensland, and from the Admiralty to the Commodore of the Australian station.

I arrived in Australia at the end of September, 1883, and for various reasons determined to make Sydney, N.S.W., my headquarters.

I should like to take this opportunity of expressing my thanks to the Ministers of the Colonial Governments, especially to the Right Honourable W. BEDE DALLEY, of Sydney, and to Sir SAMUEL W. GRIFFITHS, of Brisbane, for the assistance and facilities afforded me. The President of the Linnean Society of New South Wales, the Honourable WILLIAM MACLEAY, gave me the use of a temporary laboratory, which I occupied until the Government gave me a more convenient set of rooms in the beginning of 1884. This is only one of many kindnesses for which I am indebted to Mr. MACLEAY.

Guided by Dr. BENNETT's observations, I had intended to go after *Ornithorhynchus* immediately on my arrival, but unfortunately I wasted a fortnight trying to obtain

information in Sydney as to where the animals were to be found in sufficient numbers for my purpose. On October 12 I started for the colder districts in New England, but very soon discovered that no uterine stages were to be obtained during that season. I found, however, that the Marsupial *Phascolarctos* was just beginning to breed, and determined accordingly to collect a series of stages. W. F. GORDON, Esq., kindly invited me to stay with him at Manar, near Lake George, where *Phascolarctos* is exceedingly numerous. In December I also obtained many uterine embryos of *Halmaturus rufus*, as well as a few of other species. I returned to Sydney early in January, engaged a laboratory assistant, M. ÉMILE ROGER, and soon fitted up as a laboratory rooms given me by the Government in Macquarie Street.

During February and March I made short expeditions in search of Marsupial material, and sent home an account of the unique condition of the foetal membranes, hitherto overlooked ('Quart. Journ. Microsc. Sci.,' vol. 24, 1884).

Towards the middle of April I went north to the Burnett River to find *Ceratodus*. On the 23rd I obtained males with ripe spermatozoa. Both *Ornithorhynchus* and *Echidna* seemed to be very numerous in the Burnett district; I decided, therefore, to remain there until the Monotreme breeding season was over, in the hope of getting both *Ceratodus* and Monotremes in the same year. The Burnett district presented the further advantage of still possessing a considerable number of black natives. I afterwards found that without the services of these people I should have had little chance of success, and it soon became clear that, in order to work with them, I should have to live under canvas and carry sufficient provisions for an independent camp.

As there were still (May) many weeks of winter to pass before *Echidna* would breed, I visited Gayndah, the only inland township on the Burnett River, with the object of offering a money reward of £10 to anyone who would show me *Ceratodus* spawn. Soon after, I returned to Dalby to purchase a buggy, horses, and camp outfit, and made a flying visit to Sydney to obtain material for a prolonged collecting expedition.

During part of June and July I spent many hours daily in the water, hunting everywhere for the eggs of *Ceratodus*. Towards the end of July the blacks began to collect *Echidna*, and very soon I had segmenting ova from the uterus. In the second week of August I had similar stages in *Ornithorhynchus*, but it was not until the third week that I got the laid eggs from the pouch of *Echidna*. In the following week (August 24) I shot an *Ornithorhynchus* whose first egg had been laid; her second egg was in a partially dilated *os uteri*. This egg, of similar appearance to, though slightly larger than, that of *Echidna*, was at a stage equal to a 36-hour chick. On the 29th I sent in the telegram "Monotremes oviparous, ovum meroblastic" to a neighbouring station, where it would meet the passing mail-man, addressed to my friend Professor LIVERSIDGE, of the Sydney University, asking him to forward it to the British Association at Montreal. Meanwhile I had never relaxed my efforts to find *Ceratodus*; but after four months I was beginning to despair of success. Early in

September I had intended to go further south, so as to continue collecting *Ornithorhynchus*, but almost immediately after making this decision I found the long looked-for eggs of *Ceratodus*. This discovery changed my plan of following *Ornithorhynchus* into colder districts. The exceptional drought of 1884, by reducing the area available for spawning, made the season a particularly favourable one for my work. The next three months were spent in hatching and rearing stages of *Ceratodus*.

During this time the number of my black retainers had increased to upwards of fifty. The women were employed in searching the river weed for *Ceratodus*, while the men collected *Echidna*. It was only occasionally, and then with great difficulty, that I persuaded them to dig for *Ornithorhynchus*. Not only the blacks, but their dogs, refused to eat the animal; *Echidna*, on the other hand, was their favourite food, and their skill in finding them was very remarkable.

The result of this expedition, which had lasted from April to December, was that I had obtained many of the Monotreme stages, and a very complete series of *Ceratodus*; but I recognised that my camp had not been organised on a sufficiently large scale. In order to obtain a complete series of Monotremes, another season was necessary, and a very much larger camp.

My work on the Burnett River was greatly assisted by the hospitality of the squatters. I am particularly indebted to W. F. McCORD, Esq., and GEORGE MUNRO, Esq., who at all times rendered invaluable assistance, both by lending me horses and men, and allowing me temporary supplies from the station stores.

In December I returned to Sydney, with six clear months free for collecting Marsupial embryos, before the Monotremes would begin to breed again. The extreme delicacy of the early stages of Marsupials made it impossible to preserve them perfectly while travelling. The only satisfactory method was to be present at a large Kangaroo drive, where great numbers are driven into a small yard. The Marsupial Acts and the dry seasons had so thinned their numbers that few stations found it profitable any longer to drive the animals; the system of paying so much a head to independent hunters had replaced the yards. Fortunately, through the kindness of some squatters in the Gwydir district, a drive was arranged, and I had the good fortune to get over 80 uterine stages of *Macropus major*, besides several of other species.

From January 1 to the middle of March I travelled in a light buggy, collecting Marsupials. At the end of this time an accident deprived me of the results of this expedition. Crossing the MacIntyre River in a flood, the buggy was upset, and its contents washed away. The two following months were lost through the effects of a fever which I had contracted in the swamps of the Burnett River, and it was not until June that I started to organise the large camp of blacks, to continue the delayed attack on the Monotremes. With the help of my friend, REGINALD BLOXSOME, Esq., over 150 natives were employed during July and August, and they caught between

1300 and 1400 *Echidna* of both sexes, from which a fairly complete series of stages was obtained. A skilful black, when he was hungry, generally brought in one female *Echidna*, together with several males, every day. The former seemed to be much more difficult to find than the latter at this season. The blacks were paid half-a-crown for every female, but the price of flour, tea, and sugar, which I sold to them, rose with the supply of *Echidna*. The half-crowns were, therefore, always just sufficient to buy food enough to keep the lazy blacks hungry. The supplies were carried on a six-horse dray, and the light buggy with four-in-hand proved very much more convenient than the heavier trap of the year before. In September my friend BLOXSOME superintended the transfer of the camp to the colder river Mole, further south, where we hoped to dig out the later stages of *Ornithorhynchus* from their nests. I employed some white navvies, who opened up a large number of burrows; but the renewed exposure in Queensland had brought on my fever again, and this seriously interfered with the completion of the *Ornithorhynchus* series.

The later stages of Monotreme development have, therefore, to be worked out, mainly with *Echidna* material.

PREVIOUS INVESTIGATIONS.

Before entering on the embryology of the Monotremes, it will be, perhaps, interesting to trace the history of opinions held concerning them during the long period of ninety-two years which elapsed from their discovery in 1792 until the complete series of the stages in development were found in 1884. Nearly all the different views held concerning the nature of the female product in Monotremata have been based on indirect evidence, derived from the anatomy of the adult.

SHAW (53 and 54),* who described *Echidna* in 1792, and *Ornithorhynchus* in 1799 (*Platypus*), classed them with Edentata.

BLUMENBACH (13), after studying the skull in 1801, suggested that they might prove to be oviparous (14). Sir EVERARD HOME (35), in the same year, relying chiefly on the absence of mammary glands and the presence of a cloaca, compared them with the ovi-riviparous Sharks and Reptiles. In 1803 M. ÉTIENNE GEOFFROY SAINT-HILAIRE (19) proposed the name "Monotremata," without definitely assigning them a place in the zoological series. Between 1802 and 1819 no new facts were discovered, and in the text-books the animals were classified, either as Mammals, between Edentata and Rodentia (DESMAREST, 1804), or as forming an appendix quite at the end of the Mammalian series (TIEDEMANN, 1808; ILLIGER, 1811), or as outside the Mammalia, and "very probably" oviparous (LAMARCK, 1809). In 1812 DE BLAINVILLE (9) pointed out the resemblance to Reptilia, presented by the urino-genital apparatus and the shoulder-girdle; but he considered the resemblances to Mammalia, and especially to Marsupialia, much more marked. In 1817 CUVIER and OKEN still kept the

* The numbers in parentheses refer to the alphabetical List of Works at pages 480-484.

animals among the Edentata, but used GEOFFROY'S term, Monotremata. OKEN suggested that the mammary glands might occupy some unusual position, and so have escaped notice. In 1817, also, Sir JOHN JAMISON (38), writing from Australia to the Secretary of the Linnean Society, described the spur of the male, and stated that the female was oviparous. Sir EVERARD HOME (37), in 1819, discovered that the left ovary alone was functional, and that in the ovary the egg-follicle contained a large quantity of yolk, like a Bird's.

In 1820 Mr. PATRICK HILL, Surgeon, R.N. (34), made some observations which caused him to write to the Linnean Society; the letter was dated January 3, 1821, but was not read until December 18. Mr. HILL describes how he found an "egg in the left ovarium," "of the size of a pea, and of a yellow colour." This specimen was sent to England by a Mr. SCOTT, examined by Sir EVERARD HOME, and afterwards presented to the University of Oxford. This ovarian follicle of HILL'S gave rise to considerable misunderstanding; thus FLEMING, in his 'Philosophy of Zoology,' 1822, p. 215, stated that *Ornithorhynchus* eggs had been transmitted to London. Recently, BEDDARD (2) has quoted FLEMING, but omitted to point out that the latter knew that the egg referred to had not been laid, as FLEMING added, "It would be interesting to know the manner of incubation, and whether oviparous or ovoviviparous." In the 'Isis' for 1823, col. 1425, OKEN, after printing GEOFFROY'S remarks, and HILL'S letter on which they were based, disposes of the "celebrated history of the *Ornithorhynchus* egg" by pointing out the Mammalian characters of HILL'S Graafian follicle. OKEN concludes by saying that the whole story arose from the mammary glands not having been observed.

In 1823, however, MECKEL (40) discovered mammary glands, and his great work (41) was published in 1826. MECKEL, while rejecting both HILL'S evidence and OKEN'S arguments, pointed out that there was little difference between viviparity and oviparity, since Birds had been artificially made to incubate their eggs in the oviduct. GEOFFROY had by this time become convinced that the animals were oviparous, and he soon denied (22) that MECKEL'S glands were mammary. In 1829 GEOFFROY obtained, through Professor GRANT, the egg-shells which had been sent from Australia as veritable *Ornithorhynchus* eggs. GEOFFROY (23) figured the egg, but soon afterwards discovered that it was too large for *Ornithorhynchus*. In 1827 GEOFFROY (22) had stated that the diameter of the pelvis was not more than 5 lines. The reputed egg measured $1\frac{3}{8}$ inch \times $\frac{5}{8}$ inch. GEOFFROY'S first hypothesis, 1827 (22), was that the egg was formed in the cloaca, but so rapidly as not to cause a serious obstruction! His second, 1833 (27), was that the egg remained in the oviduct until hatching, because it could not pass through the small pelvis.

In 1832 the first direct evidence bearing on the question was published in the 'Proceedings of the Committee of Science and Correspondence of the Zoological Society of London.'

Lieut. the Hon. LAUDERDALE MAULE (39) wrote to his friend Dr. WEATHERHEAD

that he had tried to discover the grounds for the generally-accepted belief that *Ornithorhynchus* both laid eggs and suckled its young. He found in females "eggs of the size of a musket-ball, but without the hard outer shell, and, in the nest, young ones, and remains of a substance resembling egg-shell."

Three young *Ornithorhynchi*, found by Lieut. MAULE in the nest, were sent home to Dr. WEATHERHEAD; two of these were given to Sir R. OWEN, the third was given to M. GEOFFROY. Sir R. OWEN (45) discovered in these the caruncle on the bill. This striking structure, however, only confirmed OWEN in his belief in ovi-viviparity. He wrote, 1834,* the Marsupialia and Monotremata "may be regarded as an aberrant group of Mammalia, characterised by an ovo-viviparous generation."

Sir R. OWEN's researches were made possible by the energy of Dr. GEORGE BENNETT, who himself made several expeditions with the object of finding the early stages of *Ornithorhynchus*. Dr. BENNETT (3, 4, 5, and 6) has accumulated a large number of interesting observations on the habits of the animal, and has never ceased trying to furnish Sir R. OWEN with the material necessary for solving the problem of Monotreme generation.

The next direct observation was made in 1847 by M. JULES VERREAUX (55) in Tasmania. He writes:—"Le nombre d'*Ornithorhynques* que j'ai possédé m'a parfaitement démontré que cet animal ne pond pas d'œufs, mais qu'il est ovo-vivipare."

Sir R. OWEN (47) reviewed M. VERREAUX's paper in 1848, and stated that he had calculated the period of uterine gestation at about six weeks! In a foot-note, p. 318, Sir R. OWEN, contradicting a statement in CARPENTER's 'Human Physiology,' to the effect that there was no positive evidence in favour of the ovi-viviparity of Monotremes, says, "The minute size of the ovarian ovum, and consequently of the vitellus; the presence of small ova, with a delicate chorion and without chalazæ or shell, in the uterine portion of the oviduct; the absence of any shell-forming portion of the oviduct,—all are elements of a body of positive evidence in favour of the ovi-viviparity of the *Ornithorhynchus*, which needs only the discovery of the fœtus *in utero* for decisive confirmation."

In 1864 Mr. GEORGE RUMBY (52), a gold-receiver in Australia, obtained from some miners a living female *Ornithorhynchus*. This animal, shut up in a gin-case, laid two eggs which were "white, soft and without shell, easily compressible, and about the size of a crow's egg." Mr. RUMBY wrote in a letter that these eggs might be abortions caused by fear, and this suggestion was evidently accepted by OWEN, who published the letter in 1865 (48). These eggs were also seen by Dr. JOHN NICHOLSON (42), who wrote directly to OWEN about them (48). Sir R. OWEN in this paper, 1865 (48), describes a mammary fœtus sent to him by Baron FERDINAND VON MUELLER and Dr. RUDALL, and, in discussing the uterine ova, describes the "smooth chorion as being firmer than that of Rodentia; whence, and for other reasons (*vide* 'Phil. Trans.,' 1834)," he still considered the animals ovi-viviparous.

* 'Phil. Trans.,' 1834, p. 356.

From 1875 to 1883 Dr. GEORGE BENNETT's son (7, 8) collected material for Sir R. OWEN in Queensland. In 1880 Sir R. OWEN (49) described and figured several ova of *Echidna*, taken from the uterus. In the largest of these, measuring 6 mm. in diameter, he observed an artificially-produced furrow, which he described as the first furrow of segmentation, mentioning this fission of the germ as "additional evidence of the *viviparity** of the Monotremes."

In 1882 Sir R. OWEN found, lying free in the uterus of an *Echidna*, a large ovum with a "thick chorion"; *vide* paper published in 1884 (50). Mr. HAACKE (32, 33) found, on the 25th of August, 1884, an egg-shell, the remains of a rotten egg, in the pouch of *Echidna*, and showed it to the Royal Society of South Australia on the 2nd of September.

PART I.—THE EGG MEMBRANES, AND THE OVA UP TO THE FIRST STAGES OF SEGMENTATION.

Part I. of my studies contains an account of the egg membranes, and the development of the ovum up to the first stages of segmentation, in Monotremata and Marsupialia. This limitation will enable me to trace the gradual replacement of ovarian food-yolk by uterine nutrition through the Mammalia.

The comparison of the egg membranes yields a striking phylogenetic interpretation of their development.

The few previous investigators have failed to trace the egg membranes.

Sir EVERARD HOME, Mr. HILL, Sir R. OWEN, and M. VERREAUX have described the appearance of the ovary in Monotremata. The two first observers pointed out the large size of the Graafian follicle, and Sir EVERARD (37) figured the yellow yolk they contained, but Sir R. OWEN, in 1834 (44), still considered it probable, on *a priori* grounds, that the Monotremes had a small ovum, like other Mammalia.

More recently POULTON (51), BEDDARD (2), and GULDBERG (31) have observed the large size of the ovarian ovum, the eccentric position of the germinal vesicle, and the fact that the ovum completely fills the follicle during the whole period of ovarian life.

POULTON and GULDBERG have wrongly stated that the follicular epithelium remains always a single layer of cells.

BEDDARD figures the true condition, but agrees with the other two observers, ascribing the appearance of his preparation to bad preservation. POULTON came to the conclusion that it was probable that segmentation would be found to be unequal, perhaps partial. GULDBERG was unaware, when he wrote, that I had already described the partial segmentation. GULDBERG and BEDDARD lost all trace of the vitelline membrane in late stages. The former says the follicular epithelium becomes changed into a chorion:—"es scheint, als ob die Zellen verschmolzen sind, um eine helle, schwach tingir-

* I have added the italics, because the quotation shows the value of the old terms "*ovi-viviparous*" and "*viviparous*."

bare Membran zu bilden." This statement is of interest in connection with SELENKA'S (57) similar account of the formation of an egg membrane in the Opossum. The ovary of Marsupialia has been described and figured in several genera. Sir R. OWEN observed the enormous stalked follicles of *Phascolomys*. POULTON'S account of the development of the Graafian follicle in *Phalangista* is the only paper on the subject known to me. He noticed that the cumulus proligerus separated from the wall of the follicle. SELENKA (57) found follicular cells in the Fallopian tube of the Opossum. Observations on the uterine egg membranes of Monotremata have been made by Sir R. OWEN, who in 1865 distinguished two membranes, an outer "chorion" and an inner "membrana vitelli," but he wrongly stated that the "increase of the size of the uterine over the ripe ovarian ovum was due to an increase of fluid between the chorion and the membrana vitelli." SELENKA is the only observer of the early uterine stages of the egg membranes of Marsupialia. He derives the outer membrane of the uterine ovum directly from the follicular epithelium, and further states that the zona pellucida soon disappears, which results in his confusing the albumen layer of the early stages with the coagulum surrounding the embryo in later stages. SELENKA, in his preliminary note on the Opossum (56), says, "Die Eier halten die Mitte zwischen den meroblastischen und holoblastischen." The exact meaning of this statement is made clear by the beautiful drawings in his Memoir.

A.—THE EGG MEMBRANES.

1.—*Monotremata*.

i. *In the ovary*.—Round the very young ova a fine membrane is present between the single row of follicular cells and the ovum. I shall speak of this as the vitelline membrane, but whether it is produced by the ovum or the follicular epithelial cells I have not attempted to determine.

With the growth of the ovum the vitelline membrane increases enormously in thickness. In ova of .32 mm. diameter it reaches its maximum thickness of .016 mm. With the formation of the yellow yolk spheres the vitelline membrane again becomes thinner, till in the ripe ovarian ovum it has no longer a measurable thickness. The vitelline membrane is perforated by protoplasmic processes connecting the protoplasm of the ovum with that of the follicular epithelium.

Round very young ova the follicular epithelium consists of a single layer of flattened cells, each of which, like the ovum itself, consists of clear protoplasm nearly free from granules.

The difference between a ripe ovum and such an unstainable cell is caused by the addition of food material, which is formed in the cells of the follicular epithelium as well as in the ovum itself, and appears first as minute granules in the neighbourhood of the nuclei and germinal vesicle. Every stage is to be found, from the most minute granules up to the largest yellow yolk spheres of the ripe ovum.

From the nucleus of each follicular cell and from the germinal vesicle, streams of these yolk granules travel into the body of the ovum. The result of this double mode of origin of the yolk is that the yolk is interrupted at one place, viz., where the germinal vesicle lies. The yolk in Monotremata has the same arrangement as that of Birds, where a central bottle-shaped mass of smaller spheres is continued from the germinal disc to the centre of the ovum. The streams of yolk granules passing into the ovum, immediately on entering, are more or less parallel to each other, and give a radially striated appearance to the peripheral layer of the ovum (Plate 29, fig. 1). A similar appearance in other Vertebrate eggs has been described as a definite membrane, and named the zona radiata. The time during which the greater number of yolk granules are formed in the cells of the follicular epithelium corresponds with a marked change in the appearance of the layer itself. When the ovum has reached a diameter of .2 mm. the cells of the follicular epithelium, already much more columnar than in the younger stages, divide rapidly and form a layer three to four cells deep (Plate 29, fig. 1, *fe.*). When the ovum has increased to .5 mm. diameter it has received from the follicular cells such a number of yolk granules as would be nearly sufficient, if the granules were swollen to yellow yolk spheres, to completely fill a ripe ovum measuring 3 mm. in *Echidna* and 2.5 mm. in *Ornithorhynchus*. Between the stages of .32 mm. diameter and .5 mm. diameter the follicular epithelium again becomes one cell deep. This change corresponds with the change in the manner of growth of the yolk. Up to stage .32 mm. the formation of new granules was the chief function of the epithelium. After the granules are formed the absorption of fluid by osmosis becomes relatively more important; consequently, the yolk granule forming period may be conveniently spoken of as the first period of the activity of the follicular epithelium, while the period of absorption of fluid may be described as the second period.

Both processes, of course, go on during the whole of both periods. Each period includes the maximum activity of one of the two processes, granule formation or absorption of fluid.*

The active change of granules into yellow yolk spheres by the absorption of fluid is marked by the return of follicular epithelium to the condition of a single row of cells.

Plate 29, fig. 2, shows the condition of the follicular epithelium (*fe.*) and the vitelline membrane (*vm.*) in an ovum measuring 1 mm. diameter.

The follicular epithelium becomes so flat that BEDDARD (2), who has recently described the ovary of *Echidna*, failed to find it; while GULDBERG, as has been already mentioned, stated that the follicular cells fused together to form a clear "chorion."

When the ovum has reached its maximum diameter the follicular epithelium again wakes up; and the cells increase enormously in size, and each nucleus becomes larger than an entire cell in the previous stage, Plate 29, fig. 3 (*fe.*). This renewed activity

* It is to be understood that this account of the origin of yolk is only a description of the structural appearances presented by preserved material. The physiological changes may be much more complicated.

of the follicular epithelium constitutes a well-marked stage, and will be described as the third period.

The follicle has by this time come to project from the ovary so much that, in many cases, there is a marked pedicle of attachment. The follicular epithelium, beginning in the region underlying the projecting surface of the follicle, divides rapidly, and the cells, becoming enormously enlarged, secrete a dense homogeneous substance on their inner face, next the vitelline membrane (Plate 29, fig. 4, *ch*). This process of secretion goes on until the whole egg is suspended in a dense layer, which, from its fate, I shall speak of as the "pro-albumen." The follicle, being now clasped by the open mouth of the Fallopian tube, bursts, and the egg is received into the Fallopian tube.

A few cells of the follicular epithelium remain attached to the pro-albumen. The majority of the follicular cells remain behind, inside the follicle, and there they multiply so rapidly that very soon the whole cavity of the follicle is occupied by gigantic cells derived from continued division of the follicular epithelium, between which connective tissue cells have also grown in from the walls of the follicle itself. The further changes that take place in this "corpus luteum" need not be described.

ii. *In the Fallopian tube.*—I found one female *Ornithorhynchus* with two eggs in the dilated end of the Fallopian tube (infundibulum). Both eggs had begun to segment, and one had already acquired eight segmentation nuclei. Each egg was enclosed by its thin vitelline membrane (Plate 30, fig. 1; Plate 31, fig. 3, *vm.*), and surrounded by the pro-albumen. Here and there traces of the follicular cells, which remained attached to their own secretion, are visible in the sections. A few darkly staining granules were visible all round the ovum, connected with each other by a fine, darkly staining line, just inside the vitelline membrane. This layer was already visible in some sections through ripe ova, after the pro-albumen was formed. The unsegmented egg of *Echidna* (a medium section through the germinal disc of which is figured on Plate 31, fig. 1) was taken from the lower part of the Fallopian tube. In this egg the vitelline membrane has already increased in thickness to about .0016 mm., and the pro-albumen has also increased in thickness until it becomes the definite albumen layer by absorption of fluid in the Fallopian tube. In the living egg this albumen has the same appearance as the albumen of a Hen's egg, and, treated with alcohol, it comes down as a granular precipitate. (Plate 30, fig. 3, *alb.*; Plate 31, fig. 1, *alb.*) The albumen in hardened ova varies in thickness at different places by reason of its fluid nature. That the section figured in Plate 30, fig. 3, happens to have less thickness of albumen than the pro-albumen from which it arose is explained in this way.

Outside the albumen there is now a new structure—the shell membrane (Plate 31, fig. 1, *sh.*)—which first appears in the lower part of the Fallopian tube. The shell membrane is of a tough parchment-like consistency, and does not stain with hæmatoxylin or borax carmine. On the outside it has a roughened surface, which in section (Plate 31, fig. 1, *sh.*) is seen to be caused by the presence of numerous fine

villi. I have not yet tried to trace the deposition of the shell to any special glands. From the fact that these villi become longer as the egg increases in size it is clear that the shell does not thicken at the expense of the albumen layer inside.

iii. *In the uterus*.—The egg, arrived in the uterus, has already received its full complement of membranes. The albuminous investment and the shell have added 1.5 mm. to the egg's diameter. The egg continues to increase in diameter after its arrival in the uterus, and by the time it has reached a diameter of 6.5 mm. the albumen layer has entirely disappeared, and the vitelline membrane thus comes to lie close to the shell. (Plate 30, figs. 3 and 4.) Meanwhile both shell membrane and vitelline membrane have again increased in thickness. (Plate 30, fig. 2, *sh.* and *vm.*) Another layer, which stains darkly, present in the ovary after the pro-albumen appeared as the very delicate line containing granules, already described, lies inside the vitelline membrane. I look on it as a coagulum formed by reagents from the nutritive fluid entering the ovum. In the uterus it becomes very conspicuous on the disappearance of the albumen, and its presence enables the opening of the blastopore to be traced.

The egg, on leaving its follicle, measured 2.5 mm. to 3 mm. in diameter. When it is laid it measures 15 mm. by 12 mm.*

This enormous increase is due to the absorption of fluid from the walls of the uterus. The process shows itself by the continually increasing quantity of darkly staining coagulum inside the vitelline membrane and through the body of the yolk. Up to the close of segmentation this layer is thicker over the blastoderm than elsewhere. In many sections mounted in balsam the line between the vitelline membrane and this coagulum is not distinct, and the two appear as one thick vitelline membrane.

The fluid layer betrays its nature, however, by passing in between the cells, and into an opening of the blastopore.

The earliest stage of the shell membrane has been already described. Some of the stages it passes through before being laid are shown on Plate 30 (figs. 2, 3, and 4, *sh.*). In *Echidna* I have not detected any calcic salts in the shell after laying, but on treating the shell of *Ornithorhynchus* with dilute hydrochloric acid a considerable quantity of gas is given off. When fresh-laid, the egg has a thickness of .5 mm. and is of an opaque white colour; the cones figured on Plate 30 (fig. 4, *sh.*) are directly derived from the fine villi on the outside of the young shell membrane.

2.—*Marsupialia*.

i. *In the ovary*.—The development of the membranes just traced in Monotremata proceeds in exactly the same way in *Phascogale* up to the stage when the yolk granules begin in the Monotremata to become the characteristic yellow

* The laid eggs of both *Echidna* and *Ornithorhynchus* vary somewhat in size. I have a normal *Echidna* egg as small as 13 mm. by 12 mm.

yolk spheres (compare Plate 29, fig. 1, with Plate 29, fig. 5). In *Phascolarctos*, as in Monotremata, the delicate membrane surrounding the youngest ovum gradually changes into a distinct and strong membrane surrounding the ripe ovum. The ripe ovum of *Phascolarctos*, measuring .17 mm., resembles an ovum of *Echidna* or *Ornithorhynchus* measuring .25 mm. The follicular epithelium throughout the ovarian period is connected with the ovum by numerous processes perforating the vitelline membrane, along which the yolk granules pass into the ovum.

The youngest ova of *Phascolarctos* exactly resemble the youngest already described in Monotremata. The single layer of follicular cells soon becomes columnar, and also, by division, several rows deep. A cavity appears between the cells, in the same way as in Placentalia, and soon a typical Graafian follicle with its "discus proligerus" is formed.

The follicle of *Phascolarctos* grows very much larger than in most Marsupialia. In *Phascolarctos* the ripe follicle is in many cases attached to the ovary by a very slender pedicle. The follicle is elliptical in shape, and measures 10 mm. by 7 mm. It is thus enormously larger than the ripe follicle of Monotremata.

The split in the layers of the follicular epithelium, which forms the cavity of the Graafian follicle, extends completely round the ovum, so that the ovum, still surrounded by the follicular epithelium several cells deep, now lies in the centre of the follicle, with the so-called "liquor folliculi" on all sides of it. The "liquor folliculi" contains numerous branched cells, connecting the epithelial lining of the follicle with that surrounding the ovum (Plate 29, fig. 5, *fe.*).

ii. *In the Fallopian tube.*—I have no observations on the membranes in the Fallopian tube of Marsupials. The few young ova I obtained were used for other purposes. I was not aware of the existence of the pro-albumen at the time I found them.

iii. *In the uterus.*—The ovum of *Phascolarctos* passes rapidly into the uterus, and the ovum figured on Plate 29, fig. 5, had only reached the stage of the first furrow of segmentation. In this ovum the same membranes are present as in the corresponding stage of *Echidna*, with the striking difference that the follicle cells are still attached to a dense layer, similar to the pro-albumen in the Fallopian tube of Monotremata. This layer (Plate 29, fig. 5, *ch.*) lies immediately outside the vitelline membrane. Imbedded in it, and lying on the outside of it (Plate 30, fig. 5, *fe.*), are a large number of follicular cells, and, although their nuclei and nucleoli are still present, and suffice to prove their cellular nature, their general appearance, spherical form, and the absence of connecting processes between the cells, show that they are breaking down.

Though I have failed to trace the formation of this layer in Marsupials from the follicular epithelium, its structure, position, and history all point to its being homologous with the pro-albumen of Monotremata. Towards the outside the cells and the pro-albumen are surrounded with fluid (Plate 30, fig. 5, *alb.*), and the outer part of the pro-albumen itself is less dense than the inner.

Enclosing the whole egg is a thin transparent membrane .0015 mm. thick, similar

to the shell membrane of Monotremata (Plate 30, fig. 5, *sh.*). The further changes undergone by these membranes enclosing the ovum are as follows:—The ovum soon increases in size, and in an ovum measuring .3 mm. the shell has become markedly thicker, viz., .01 mm. The vitelline membrane also increases slightly in thickness, but the great increase in size of the ovum is due to the swelling up of the follicular secretion by the absorption of fluid from the walls of the uterus (Plate 30, fig. 7, *alb.*). Two stages are shown in section of this swelling up (Plate 30, figs. 6 and 7). All traces of follicular cells have vanished by this time. The further changes that take place with the formation of the blastodermic vesicle and development of the yolk sac are similar to those that occur in Monotremata at a corresponding age. The albumen soon disappears, and the vitelline membrane comes to lie close to the shell. No sooner is the albumen layer formed than it begins to pass through the vitelline membrane to nourish the ovum, and after the albumen layer has disappeared another layer has become very conspicuous inside the vitelline membrane. This layer is a coagulum formed from nutritive fluid on its way to feed the developing embryo, and shows its fluid nature, in the same way as pointed out in Monotremata, by passing into the open portion of the blastopore, and between the cells of the blastoderm. The distinction between the vitelline membrane, now considerably increased in thickness, and this coagulum is difficult to trace all round the ovum.

This fact has led SELENKA to describe the vitelline membrane as disappearing at an early stage, and the albumen as being directly continuous in development with the coagulum. The membrane, composed of the shell and the vitelline membrane, persists up to the stage when the blastodermic vesicle becomes fixed to the walls of the uterus. It is possible that it may persist for a longer period over the non-vascular unattached area of the yolk sac and allantois (*vide* my paper, 'Quart. Journ. Microsc. Sci.', 1884). The blastodermic vesicle enclosed in the membrane just described, measuring 15 mm. in diameter, floats freely in the uterus, and is exactly comparable to the laid egg of Monotremes. I have not found any trace of villi on the surface of the shell in Marsupials.

B.—THE DEVELOPMENT OF THE OVA UP TO THE FIRST STAGES IN SEGMENTATION.

Monotremata and Marsupialia.

Some of the changes that take place in the ovum have been described in dealing with the follicular epithelium. The ovum, while maintaining its character of a single cell, has become loaded with food material, which has been deposited in a horse-shoe-shaped mass round the germinal vesicle. This horse-shoe-like arrangement of the yolk is caused by its double mode of origin, and is common to all Vertebrata with yolk-forming follicular epithelium. The different positions in which the yolk has been described as arising in meroblastic eggs may not be so different as has been supposed. At the end of the first period in Monotremata the yellow yolk spheres

appear as such at considerable distance from the surface of the ovum. In the ripe egg there is only a very thin layer of fine granules and white yolk, between the yellow yolk and the vitelline membrane. This distribution appears to be due to the relative rapidity the formation of yolk granules bears to their absorption of fluid at any one period. The active protoplasm of *Phascolarctos*, like that of Monotremata, is aggregated to one pole of the ovum. There is a lens-shaped germinal disc in *Phascolarctos* as in Monotremata (Plate 29, fig. 5, and Plate 31, fig. 1).

The yolk of *Phascolarctos* never gets beyond the stage of white yolk. The white yolk immediately below the germinal vesicle in Monotremata is the only part of the ovum where oil (?) globules occur (Plate 31, fig. 1). The whole yolk of *Phascolarctos* is similar to the region below the germinal disc of Monotremes. The details of the segmentation and the exact behaviour of the cells to form the gastrula will be traced in Part II. I shall here only describe the general characteristics of segmentation in Monotremata and Marsupialia, and point out how the segmentation of Placentalia has been derived from this. The first furrow marks out the germinal disc into a larger and a smaller area (Plate 30, fig. 5, n_1 and n_2). The first furrow has therefore made the ovum bilaterally symmetrical. The second furrow appears at right angles to the first, and divides the germinal disc into four regions, two larger and two smaller (Plate 31, fig. 2, n_1 , n_2). All these regions are connected with each other and with the yolk by protoplasmic processes passing across the furrows. These first four nuclei give rise by division to all the nuclei of the future embryo. No nucleus of the yolk which would explain spontaneous formation of "yolk nuclei" is present. So far the description applies equally to *Phascolarctos* and to Monotremata, but the ovum of *Phascolarctos* is no larger than that of Placentalia.

That the first two segmentation furrows should fail to divide the ovum, shows that although the ovum has nearly regained its original alecithal condition, it still retains the secondary arrangement of protoplasm, induced by the yolk of its more immediate ancestors.

Comparison of the Egg Membranes of Monotremata and Marsupialia with those of Placentalia.

From the detailed account of the development of the egg membranes in Monotremata and Marsupialia (*Phascolarctos*) it is clear that there are two primary egg membranes in each group, the vitelline membrane and the pro-albumen, while a secondary egg membrane, the shell membrane, is added in the Fallopian tube and uterus.

There is a strong presumption that the egg membranes of Placentalia are homologous with those of Monotremata and Marsupialia. I shall attempt to show that the three membranes I have found in Monotremata and Marsupialia are present in Placentalia.

There are two egg membranes generally recognised in Placentalia, the zona pellucida and the vitelline membrane.

This vitelline membrane was first noticed by VALENTIN; BARRY (60) figured it in 1838, but BISCHOFF (66) immediately afterwards explained that BARRY and the previous observers had been misled by a line caused by the existence of cilia round the young ovum.

H. MEYER (72), REICHERT (73), and VAN BENEDEN (63) have distinguished this structure, and regarded it as a true vitelline membrane. HEAPE (70) found and figured it in sections of the ripe ovum of the Mole.

BARRY stated in his papers that it disappeared by liquefaction, but in a foot-note in p. 338 in his last paper he says that it "may perhaps contribute to the thickening" of the zona pellucida; and this foot-note of BARRY's is evidence confirmatory of the suggestion that I now make concerning this membrane.

VAN BENEDEN (64) says the appearance of this true vitelline membrane coincides with maturation and the formation of polar bodies.

I would suggest, therefore, that it is the homologue in Placentalia of the layer which, appearing during maturation as a line containing granules, becomes the coagulum of later stages in Monotremata and Marsupialia.

VON BAER (58) discovered the zona pellucida in the ovary. BARRY (62) figured its radial striation, caused by the pointed ends of the follicular epithelium, and its granular outer layer.

In 1854 REMAK described the radial striation, but it was not until 1870 that WALDEYER (74) called attention to the distinction between the outer and the inner layers of the zona radiata.

WALDEYER considered the granular layer a product of the follicular epithelium. BALFOUR (59) put forward a curious hypothesis with regard to this outer layer. He considered that it was the remains of the vitelline membrane surrounding young ova. BALFOUR's hypothesis was rendered necessary by his comparison of the inner zona radiata of Mammalia with the transitory appearance which he called zona radiata in Elasmobranchs.

VAN BENEDEN (65), p. 514, pointed out BALFOUR's mistake, and said, "It is certain that the membrane which BALFOUR called vitelline membrane in the Rabbit is the zona radiata, and not its outer granular layer." There is, therefore, in Placentalia, as in Monotremata and Marsupialia, a delicate membrane round the young ovum, which becomes the thick membrane known as the inner homogeneous layer of the zona pellucida. This membrane is the true vitelline membrane of Placentalia. The outer granular layer may therefore be interpreted as the homologue of the pro-albumen.

The outer covering of the uterine embryo was discovered by DE GRAAF in 1692. VON BAER (58) suggested that this "villous chorion" (*Schalenhaut*) was directly derived from the zona pellucida of the ovary.

COSTE and RUDOLF WAGNER held the same view; but PURKINJE, VALENTIN, and ALLEN THOMSON all considered that the chorion probably arose in the oviduct, like the egg-shell of other Vertebrates.

WHARTON JONES (71) was the first to notice the addition of the albuminous coating in the Fallopian tube. He considered that this coating gave rise to the chorion. BARRY (61) has given a most elaborate account of the origin of the chorion. His results are strikingly similar to what I have described in *Phascolarctos*; and it is not too much to admit that, if no one had thrown doubts on BARRY'S work, I should have had no difficulty in comparing the membranes of *Phascolarctos* with those of Monotremata and Marsupialia. Dr. BARRY (62) stated that the chorion arose from "cells" (not the cells of the tunica granulosa, but "cells" appearing in the Fallopian tube) on the outside of the zona pellucida; that this chorion imbibed fluid, and separated from the zona pellucida; that after the fluid was absorbed the zona pellucida again lay close to the chorion; and that the chorion gave rise to villi. Dr. BARRY'S experiment of crushing the fresh ovum, and finding that its contents, passing outside the zona pellucida, were still contained in his chorion, is striking evidence in favour of the existence of such a membrane, although, unfortunately, no recent observers have described any trace of it.

TABLE showing the Homologies of the Egg Membranes in Mammalia.

| | MONOTREMATA. | MARSUPIALIA. | PLACENTALIA. |
|---|------------------|--|--------------------------------------|
| Layer of coagulated fluid in hardened mature ova. | = Coagulum . . . | = Coagulum. . . | = Vitelline membrane of VAN BENEDEN. |
| Egg membranes. | Primary. | Vitelline membrane = Vitelline membrane = Vitelline membrane = Zona radiata = inner layer of mature ovary, zona pellucida. | |
| | | Pro-albumen secreted by follicular epithelium. = Pro-albumen . . . = Pro-albumen. . . = Outer granular layer of zona pellucida (WALDEYER). | |
| | Secondary. | Shell membrane = Shell membrane = Shell membrane = Villous? "non-cellular" chorion (BISCHOFF). formed outside albumen in Fallopian tube and uterus. with villi which become "cones." without villi? | |

Comparison of the Egg Membranes of Mammalia with those of other Vertebrata.

Very numerous statements have been made concerning the egg membranes of both Ichthyopsida and Sauropsida (68 and 69). I shall not attempt to compare all the membranes that have been described, because it is both certain that many are either transitory arrangements of the peripheral protoplasm of the egg or the product of hardening reagents, and also probable that some are optical illusions.

That a pro-albumen such as I have described in Monotremata has not been recognised in most Vertebrata is due possibly to the shortness of the period of its formation.

CUNNINGHAM (67), however, has recently shown that such a structure exists in *Myxina*, but he assumed that it gave rise directly to the shell, and consequently could

not be homologous with the albumen or the shell of Elasmobranchii. It seems more probable that the difference between the shell of Elasmobranchii and that of *Myxine* is due to the fact that in Elasmobranchii the pro-albumen absorbs fluid in the course of its passage down the oviduct.

The shell membrane presents another difficulty. I have shown that in Marsupialia it does not increase at the expense of the albumen.

CUNNINGHAM states that the shell of *Myxine* arises directly from the pro-albumen, and others have imagined a similar origin for it in Elasmobranchs and Sauropsida.

VON BAER compared the albumen of a Frog's egg to the shell of a Bird's.

These considerations show that further investigations are necessary in both Ichthyopsida and Sauropsida before the three membranes can be traced through Vertebrates.

SUMMARY.

In Monotremata and Marsupialia—

- I. There is a vitelline membrane which, appearing between the young ovum and the follicular epithelium, persists until hatching in Monotremata, and until late uterine stages in Marsupialia.
- II. There is a second primary egg membrane secreted by the follicular epithelium shortly before the ovum leaves the ovarian follicle—the pro-albumen.
- III. The pro-albumen, by absorption of fluid in the Fallopian tube and uterus, becomes the albumen layer outside the vitelline membrane.
- IV. A secondary egg membrane—the shell membrane—is found in the Fallopian tube, and becomes thicker in the uterus.
- V. The albumen soon disappears, and the vitelline membrane comes to lie next the shell.
- VI. The ovum absorbs fluid from the uterus, and increases in Monotremes from about 3 mm. to 15×13 mm.
- VII. A layer, simulating the appearance of a membrane, inside the vitelline membrane, is a coagulum formed by reagents from the nutritive fluid passing into the ovum.
- VIII. There is a germinal disc, and the ovum undergoes a partial segmentation in Monotremes and *Phascogaleos*.

In Placentalia—

- IX. The vitelline membrane has generally been known as part of the zona pellucida.
- X. The pro-albumen is probably represented by the outer "granular layer" of the zona pellucida.
- XI. The shell membrane has not been recognised in its early stages, except by BARRY. The "villous chorion" (non-cellular, BISCHOFF) is probably partly derived from a true shell membrane.

XII. The delicate layer immediately surrounding the ripe ovum, known as the "true vitelline membrane," VAN BENEDEN, is perhaps the first stage of the substance described as "coagulum" in Monotremata and Marsupialia.

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EXPLANATION OF THE PLATES.

Reference Letters.

- alb* = albumen.
- b* = basilar membrane of follicular epithelium
- bl* = posterior opening of blastopore.
- ch* = pro-albumen.
- ep* = epiblast.
- fe* = follicular epithelium.
- gv* = germinal vesicle.
- hy* = hypoblast.
- o* = oil (?) globule.
- n*₁ = nucleus of smaller segmentation area.
- n*₂ = „ larger „ „
- sh* = shell membrane.
- sh*₁ = middle layer of shell membrane.
- sh*₂ = papillæ of shell membrane.
- um* = coagulum.
- vm* = vitelline membrane.
- y*₁ = yolk granules.
- y*₂ = white yolk.
- y*₃ = yellow yolk.
- zr* = zona radiata.

PLATE 29. ZEISS, oc. 2, obj. $\frac{1}{18}$ homogen. : cam. luc.

- Fig. 1. *Echidna*.—Small portion of a section through the ovarian ovum, measuring .32 mm. in diameter. 1st period : *fe*, follicular epithelium ; *vm*, vitelline membrane ; *zr*, zona radiata ; *y*, yolk granules ; *y₂*, white yolk ; *o*, oil globule ; *b*, basilar membrane.
- Fig. 2. *Echidna*.—Small portion of a section through the ovarian ovum, measuring 1 mm. in diameter. 2nd period : lettering as in fig. 1.
- Fig. 3. *Echidna*.—Small portion of a section through the nearly mature ovarian ovum. Beginning of 3rd period : lettering as in fig. 1.
- Fig. 4. *Echidna*.—Small portion of a section through the ripe ovarian ovum, measuring 3 mm. in diameter. 3rd period : *ch*, pro-albumen.
- Fig. 5. *Phascolarctos cinereus*.—Medium section through a nearly mature ovarian ovum taken from the "liquor folliculi" of a follicle measuring 9 mm. \times 6 mm. : *fe*, follicular epithelium ; *vm*, vitelline membrane ; *gv*, germinal vesicle.

PLATE 30. ZEISS, oc. 2, obj. $\frac{1}{18}$ homogen. : cam. luc.

- Fig. 1. *Ornithorhynchus*. Small portion of a section through the segmenting ovum, taken from the open end of the Fallopian tube, measuring 2.6 mm. in diameter : *ch*, pro-albumen ; *vm*, vitelline membrane.
- Fig. 2. *Echidna*.—Small portion of a section through the segmenting ovum taken from the uterus, and measuring 6 mm. in diameter : *sh*, shell ; *alb*, albumen ; *vm*, vitelline membrane ; *um*, coagulum ; *bl*, blastopore ; *ep*, epiblast ; *hy*, hypoblast.
- Fig. 3. *Ornithorhynchus*.—Small portion of a section through the segmenting ovum taken from the uterus, and measuring 6 mm. in diameter : *sh*, base of shell ; *sh₁*, middle layer of ditto ; *sh₂*, papillæ of ditto.
- Fig. 4. *Echidna*.—Small portion of a section through the blastodermic vesicle, taken from the uterus, and measuring 9 mm. in diameter : *sh₂*, cones derived from papillæ of previous stage.
- Fig. 5. *Phascolarctos cinereus*.—The 17th section of a vertical longitudinal series of 35 sections through the segmenting ovum, containing 2 nuclei, taken from the uterus, and measuring .17 mm. in diameter : *sh*, shell membrane ; *fe*, cells of follicular epithelium ; *alb*, albumen ; *ch*, pro-albumen ; *vm*, vitelline membrane ; *y₁*, protoplasm, with finest yolk granules ; *y₂*, white yolk ; *n*, nucleus of smaller segmentation area ; *n₂*, nucleus of larger segmentation area.
- Fig. 6. *Phascolarctos*.—From uterus, .28 mm. in diameter, stage of 4 nuclei : *sh*, shell ; *ch*, pro-albumen ; *alb*, albumen ; *vm*, vitelline membrane.
- Fig. 7. *Phascolarctos*.—From uterus, .31 mm. in diameter ; lettering as in fig. 6.

Fig. 8. *Hypsiprymnus*.—From uterus, 4 mm. in diameter: *sh*, shell membrane; *vm*, vitelline membrane; *um*, coagulum; *bl*, blastopore; *ep*, epiblast; *hy*, hypoblast. (Cf. fig. 2.)

PLATE 31. ZEISS, oc. 2, obj. c. : cam. luc.

Four sections through the germinal disc of *Echidna* and *Ornithorhynchus*. The violet corresponds to the distribution of the more active protoplasm. The yellow yolk spheres are coloured buff.

Fig. 1. *Echidna*.—Median section through the germinal disc of an unsegmented ovum taken from the lower part of the Fallopian tube, measuring 3.2 mm. in diameter: *sh*, shell; *alb*, albumen; *vm*, vitelline membrane.

Fig. 2. *Echidna*.—Stage of four segmentation nuclei. Lateral section through one pair of nuclei made in a plane at right angles to the first furrow; ovum 4.5 mm. in diameter: *n*₁, nucleus of smaller area; *n*₂, nucleus of larger area. Other letters as in fig. 1.

Fig. 3. *Ornithorhynchus*.—From open end of Fallopian tube, 2.6 mm. diameter. Stage of eight segmentation nuclei: *ch*, pro-albumen.

Fig. 4. *Echidna*.—From uterus, 5 mm. in diameter. Median vertical longitudinal section through germinal disc: *um*, coagulum; *ep*, epiblast; *hy*, hypoblast. Other letters as in fig. 1.

XVII. *The Electromotive Properties of the Electrical Organ of Torpedo Marmorata.**By* FRANCIS GOTCH, *M.A. Oxon., B.A., B.Sc. London.**Communicated by Professor* BURDON SANDERSON, *M.D., F.R.S.*

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THE electrical organ of the Torpedo is so remarkable a structure that it would be surprising if it had not engaged the attention of anatomists and physiologists. A brief account, which may embody the principal results of their labours, is a necessary prelude to that of the present investigation.

Historical.—The discovery of the electrical nature of the shock of the Torpedo by WALSH (1)* was followed by the remarkable experiments of CAVENDISH (2).

CAVENDISH constructed an artificial Torpedo by placing charged Leyden jars in a leather case, which in size and shape resembled the actual fish, and made experiments upon this schema, in order to ascertain the influence of its surroundings—whether sea-water, wet sand, or air—upon the distribution of the electrical discharge. The results obtained were in close accord with those phenomena which prevailed during the activity of the fish, and the experiments thus confirmed in a most conclusive manner the views propounded by WALSH as to the electrical nature of this activity.

Anatomy of organ.—REDI (3) and LORENZINI (4) had described, previous to WALSH'S discovery, and termed “*musculi falcati*,” the peculiar organs which are present in the Torpedo. They rightly conjectured that these were connected with the special powers which the fish possessed, though they were quite in the dark as to the real nature of these powers. After the discovery just referred to, the structure of the fish was more fully investigated, among others by JOHN HUNTER (5), CUVIER and JOBERT DE LAMBALLE (6). In 1844 SAVI (7) published a monograph on the anatomy of the nervous system and the electrical organs of the Torpedo which gives an accurate picture of the relations of the organ to its nervous supply, and the mode of origin of its four large electrical nerves from a special central mass, the electrical lobe, situated behind the medulla oblongata, and immediately below the cerebellum.

In his splendid monograph on the more minute structure of the brain of Fish, FRITSCH (8) has described the relations of this lobe. It may be considered as a ganglionic mass forming a protuberant swelling on each side of the *calamus scriptorius*. The

* The numerals in parenthesis refer to the list of works at end.

second, third, and fourth electrical nerves are homologous to the branches of the eighth pair (vagus) in the Skate, whilst the first nerve is said to be homologous to the fifth.

Minute structure of organ.—The organ consists of hexagonal columns extending from the ventral to the dorsal surface of the animal. Each column is a connective-tissue framework enclosing a number of septa so arranged as to resemble a pile of plates in close apposition one to another. The relation of the columns to each other is shown in fig. 1, which represents a transverse section through the two organs and the body of the animal, the figure being very diagrammatic.

Fig. 1.



Transverse section of fish, with organ cut through on each side; about one-fifth natural size.

The more minute structure of the columns and septa has been investigated by PACINI, R. WAGNER, RANVIER, and others, for the details of which the work of RANVIER (9) gives ample references, and a special investigation of the termination of the electrical nerves has been made by EWALD (10). Leaving debatable matter on one side, it may be asserted that the column contains a large number of transversely disposed protoplasmic septa, in which are numerous conspicuous nuclei. The nerves enter the column in such a way that the branches lie between the septa. From these branches a very large number of finer branches proceed to the ventral surface of the septa. The physiological change, which follows the arrival of the excitatory nervous impulse, is produced almost coincidently in all the septa, and is of such a character that the ventral surface of each septum becomes negative to the dorsal surface. It must, however, be borne in mind that when examined in the fresh state no distinct surfaces are seen on the septa; the fluid, which in fresh preparations appears to intervene between the septa, being in reality the semi-fluid protoplasm of which these consist, in which Brownian movement of granules can be seen even in close proximity to the nuclei.

Physiology of organ.—If we now direct our attention to the physiological side of the subject, we find that among the numerous investigations which have been made upon the electromotive activity of the Torpedo, those associated with the names of COLLADON, MATTEUCCI, MAREY, and DU BOIS-REYMOND stand out as the most important, and the results arrived at by these observers will indicate with sufficient accuracy the present position of our knowledge.

Previous investigations.—The work of COLLADON (11), published in 1836, was passed by almost unnoticed until DU BOIS-REYMOND, in his treatise entitled "Lebende

Zitterrochen in Berlin," drew attention to it and confirmed the main statements contained in it. COLLADON determined galvanometrically the distribution of electricity on the surface of the Torpedo when the animal discharged its shock, a distribution which CAVENDISH sixty years before may be said to have forecast. The results are summed up by DU BOIS-REYMOND as follows (12) :—

"(1.) All points on the back are positive to any point on the belly. The strength of the current diminishes in proportion to the distance of these points from the organ, and almost entirely disappears at the tail.

"(2.) Any two unsymmetrical points of the back, or any two of the belly, almost always yield a current in the galvanometer. The one nearest to the organ is positive in the back, negative in the belly.

"(3.) When contact is made with two symmetrical points either of the back or belly, there is no deflection in the galvanometer.

"As COLLADON was the first to determine the electrical condition of the dorsal and ventral surfaces of the Torpedo, I have given the name of COLLADON'S currents to the currents between points on either of these surfaces."

As DU BOIS-REYMOND has shown, the distribution is further modified by the unequal length of the columns, every point of the dorsal surface of the organ being positive to a point of the same surface which is situated further away from the median edge of the organ. The reverse must naturally be the case with reference to two points on the ventral surface of the organ.

MATTEUCCI.—In 1837 MATTEUCCI (13) published his researches on the electro-physiological phenomena of animals, and devoted a large portion of his work to the electromotive phenomena manifested by the Torpedo. The earlier observations had been made upon the discharge produced in the organ on irritation of the uninjured fish. This discharge was therefore a reflex effect, the efferent path of which was clearly indicated by the electrical lobes and the large electrical nerves which proceeded from these to the organs. MATTEUCCI commenced along the same lines, and ascertained that cold abolished and moderate warmth restored this reflex discharge, that after a prolonged series of shocks the reflex was so weak as to be inappreciable, but that after an interval of rest the discharge returned with all its former intensity.

He found that a strychnised Torpedo gave a very prolonged discharge which, whilst very intense at first, grew feebler and feebler, until, as might be expected, no further effect could be detected.

Division of the electrical nerves or destruction of the electrical lobes completely abolished the discharge. He employed both the galvanometer and a much more sensitive instrument, the rheoscopic frog, for determining the presence of electromotive differences. With the nerve of a nerve-muscle preparation in contact with the surfaces of the organ, he found that a rapid electromotive change occurred in the organ on division of the electrical nerve, as evidenced by the twitch of the muscle, and that this effect occurred whenever the peripheral end of the divided nerve was excited,

whether mechanically or by the passage through it of a voltaic current. A descending direction of current was found to be more favourable than an ascending for the production of such an organ response.

The response of the organ to electrical excitation of its cut nerve thus afforded an opportunity for investigating the activity of the organ by using "nerve-organ" preparations, just as the phenomena of muscular activity had been investigated by observations made upon nerve-muscle preparations. It was, however, some years before the subject was approached along these lines.

ECKHARD.—In 1858 ECKHARD (14) showed that the observation as to the more complete response of the organ to electrical excitation of its cut nerve, when the exciting current used was a descending one, held good not only in the case of voltaic currents, but also when induction currents were used. He endeavoured with the imperfect means at his command to ascertain the character of the organ response, and came to the conclusion that its duration was longer than that of an induction shock of much the same intensity.

MAREY.—In 1871 a series of experiments was begun by MAREY (15) at Naples, which were continued at Paris (16); the full results were published in 1877, and the conclusions which he arrived at are as follows:—

(1.) Each excitation of the peripheral end of a cut electrical nerve gives rise in the organ to a single shock (*flux*), just as the similar single excitation of a motor nerve gives rise to a single contraction of the muscle which it supplies.

(2.) The time relations of the "flux," as ascertained by the frog signal, show a period of inactivity (*temps perdu*) amounting to about '01" between the excitation and the response, and a duration of the response equal to six hundredths of a second.

(3.) The reflex discharge (*décharge*) of the fish consists of a series of such single shocks. These may follow one another with great rapidity, at a rate of one to two hundred per second, and under such circumstances must blend just as successive muscular contractions fuse when the excitations which produce them occur at intervals of time which are less than the duration of the individual contractions.

(4.) The frequency and strength of the successive shocks which form the powerful reflex discharge are affected by variations in temperature, by fatigue, &c.

The experiments upon the nature of the reflex discharge were particularly ingenious; attention may be drawn to such methods, for instance, as that of leading the Torpedo discharge through the primary circuit of an induction apparatus and ascertaining by a telephone the state of the secondary coil, and as that of leading the discharge through a modified DESPREZ electro-magnetic signal and recording its movements.

JOLYET.—With regard to the time relations of the single response, it was shown in 1883 by JOLYET (17) that a different interpretation must be given to the interval which was found to elapse between the excitation of the nerve and the response of the organ. JOLYET found that a large part of the lost time was in reality consumed

in the passage of the nervous impulse down the nerve to the organ; the nerve conducting more slowly than MAREY had supposed—according to JOLYET, at a rate of 7 metres in a second.

DU BOIS-REYMOND.—The extensive work of DU BOIS-REYMOND (18) remains to be referred to. This deals not so much with the response of the organ to excitation of its electrical nerve as with the peculiar behaviour of the organ itself in respect to the passage through it of electrical currents. The main facts which are thus brought out may be broadly stated as of two kinds. First, those relating to the electromotive properties of the organ after the passage through it of an electrical current; and, secondly, those relating to the influence exerted by the organ upon the traversing current itself—in other words, phenomena of polarisation or after-effect, and of conduction.

In both these respects the behaviour of the tissue varied with the direction of the electrical experimental current. It was thus necessary to definitely label the experimental current with regard to its direction through the organ, and with this view a current passing through the tissue from the ventral to the dorsal surface was termed “homodromous,” since its direction was similar to that of the response, and a current passing in the opposite direction was termed “heterodromous.” A strong galvanic current of short duration, whether “homodromous,” or “heterodromous,” is followed by an electromotive change which shows itself galvanometrically as a current passing through the organ in the direction of the shock. With weaker galvanic currents or currents of long duration after-effects are produced which are opposed in direction to the experimental current.

The influence of the organ upon the experimental current itself is very surprising, for it would appear that the organ is a better conductor for “homodromous” than it is for “heterodromous” currents. This remarkable fact is not, according to DU BOIS-REYMOND, due to the development of an excitatory change which adds to the amount of one current whilst it diminishes that of the one oppositely directed; it can therefore only be called, for want of intelligible physical explanation, a case of “irreciprocal conduction.”

He states further that both facts are of great teleological importance, since they show that the shock of the organ, instead of being weakened by polarisation in the tissue, will be strengthened by the production of an after-effect in the same direction as itself, and that the organ is of such a character as to distinctly disfavour any short-circuiting of the current present in one column by its neighbour. To quote his own words, “the organ is not insulated, but the irreciprocal conduction which we have recognised performs, as has been already stated, a function similar to complete insulation. Each column conducts its own homodromous current comparatively well, but bars the passage to the heterodromous current threads of all the other columns; and, as this is the same for all the columns, the heterodromous current threads are forced to take the circuitous route round the edges of the organ, just as if the organ consisted of a non-conducting substance” (19).

Besides these two important facts the work of DU BOIS-REYMOND brings into notice the presence of an organ-current in the quiescent tissue (20). This organ-current is a feeble counterpart, as far as its direction and distribution are concerned, of the electromotive changes which are present during the state of activity. The existence of this current is a matter of very great interest in relation to the well-known diversity of opinion as to the so-called "demarcation" currents and "currents of rest" present in muscle and nerve.

There are thus three distinct aspects under which the electrical organ of the Torpedo has been and may be studied.

I. As an inactive organ with reference to the effect produced upon it by the operation of such agents as galvanic currents, mechanical injury, temperature, &c.

II. As an active organ, the activity of which has been evoked by excitation of the peripheral end of its divided electrical nerve, with reference to the influence of varying conditions on the time relations of this activity.

III. As an active organ, the index of a central nervous discharge.

The experiments which form the subject of this communication are concerned with the first and second aspects of the organ, the reflex discharge being reserved for further investigation at a period when the conditions should be more favourable than they were at the particular time of year (December, 1886, and January, 1887) in which the present observations were made.

Object of present research.—If we now consider to what extent our knowledge of the electromotive phenomena of the Torpedo still remains obscure, we shall be better able to appreciate the direction of the present research.

The nerve-organ response.—It has been shown that MAREY has investigated the time relations of the response of the organ to excitation of its nerve. The subsequent correction by JOLYET, previously referred to, shows that the first investigation omitted to take into consideration one most important detail. The investigations are defective in omitting other details, which, as far as the phenomena of muscle and nerve are concerned, are of great importance, such as temperature, strength of stimulus, &c. Moreover, the methods employed hitherto cannot be regarded as furnishing thoroughly reliable data. The frog-signal may be described as a touch-and-go instrument, a delicate galvanoscope which does not appreciate differences in the amount of the currents which excite it. It gives approximately accurate information as regards the commencement of a sudden electromotive change in the tissue with which it is connected, and when used, as MAREY used it, with a pendulum rheotome, may indicate the duration of that change, although it is uncertain to what extent it would respond to any slight electromotive change which may attend the subsidence of the main effect. As it gives no data from which to construct the curve which may represent the development and decline of an electromotive change, MAREY used the D'ARSPREZ signal for this purpose. The signal was modified by substituting for the usual spring an elastic cushion between the magnet and the

armature. From the curve traced by this signal when the organ response is led through it he deduces the character of the curve, developing rapidly and subsiding more slowly.

A more accurate curve might be obtained by photographing the movement of a quick capillary electrometer, but even this could not be considered satisfactory unless a series of galvanometric readings corresponding to known time intervals had been also made. The great improvement in the construction of galvanometers renders this last method still more satisfactory.

A series of galvanometric readings may not, owing to the short closures necessitated by the rheotome, give a complete account of an electromotive change, but as far as it goes it is perfectly trustworthy. It was, therefore, with the object of making such series of galvanometric readings that the electromotive phenomena of the response were re-investigated in the present research.

The organ-current.—The uncertain and meagre character of our knowledge with respect to the organ-current is much more pronounced. We are able to predict with certainty that, if in muscular or nervous tissue a violent local molecular change is effected by mechanical injury or by the application of heat or chemical reagents, a change such that there is on the one side complete death of tissue, and on the other side tissue of which the vitality is unimpaired—the former state shading into the latter—then this will manifest itself by an electromotive effect in the neighbourhood of the injury. This electromotive change is always of such a character that the impaired tissue becomes strongly negative to the unimpaired tissue.

But with regard to the possibility of producing such electromotive phenomena in the electrical organ we know nothing. The great importance of experiments upon this subject is sufficiently obvious, and the first part of the present research is, therefore, devoted to the results of such experiments. The remarkable character of the discoveries of DU BOIS-REYMOND, already referred to, warranted re-investigation, and a large number of experiments have been made with reference to these.

The results embodied in the present preliminary account may, therefore, be grouped under three heads :—

- I. Experiments relating to the organ-current.
- II. Experiments relating to the time relations of the excitatory change produced in the organ by excitation of its nerve.
- III. Experiments connected with the passage of electrical currents through the organ.

All the experiments were carried out in the months of December, 1886, and January, 1887. Through the kindness of the Société Scientifique d'Arcachon, several rooms in the Zoological Station at Arcachon were placed at the disposal of Dr. BURDON SANDERSON and myself. The methods and lines of work had to be decided upon in England, as it was necessary to take out all the essential apparatus from the Oxford Physiological Laboratory.

It thus necessarily happened that in some instances work had to be given up whilst still incomplete, as some essential piece of apparatus was wanting.

In the summer the shallow water of the Bassin d'Arcachon contains many Torpedoes; but in the winter the fish leave the basin for the deeper water outside. There was, however, no difficulty in procuring them, provided the weather was sufficiently favourable to allow the steam trawlers to work. The Torpedoes were found in sufficient numbers on the sandy bottom of the sea, at some little distance from the shore. They were caught in the trawl, and were, undoubtedly, exhausted by the process. Here, however, the cold acted favourably, for, the fish being caught at night and kept in tubs on the deck, the temperature was sufficiently low to abolish the organ reflex. When brought to the laboratory, the fish rarely showed any movement except the winking of the spiracles. Placed in water at 10°C ., they recovered, and buried themselves in the sand at the bottom of the tanks. They were easily sorted by size into small, medium-sized, and large full-grown fish. The small fish were newly-born, and measured 13 centimetres in length and 8 centimetres in breadth. Nine of these were taken from the uterus: five from one Torpedo, and four from another; the four last-named lived for one or two weeks, and were very vigorous little fish. Of Torpedoes of this class, eight were used for experiment.

The medium-sized fish were probably the last year's young. They measured from 22 to 25 centimetres in length, and from 14 to 17 centimetres in breadth. Four such fish were used for experiments.

The large fish measured from 40 to 52 centimetres in length, and from 25 to 32 centimetres in breadth; of these, seven were used for experiment.

Although only 19 fish were experimented upon, it must be borne in mind that one Torpedo furnishes a mass of material for work, since the organ preserves its excitability for many hours when kept in the cold.

I. *Experiments relating to the Organ-Current.*

The organ-current is essentially what in other tissues has been termed a "current of rest." It has been ascertained by DU BOIS-REYMOND to be present both in the entire organ and in cut-out strips of organ as a persistent effect. The current in the unexcited tissue has the same direction in the organ as that produced by the response of the organ to excitation (the shock); that is to say, it is directed through the columns from their ventral to their dorsal surfaces. Its amount, though always inconsiderable as compared with that of the response, is, like the latter, dependent upon the length of the organ columns; and, consequently, the current has the same general distribution over the surface of the organ as the excitatory effect. This is thus expressed by DU BOIS-REYMOND: "If we put out of account the smallness of the differences of potential, the electromotive surface of the quiescent fish differs from that of the same animal when giving a shock only in the induction which accompanies the shock" (21). Its presence in small strips of tissue enabled DU BOIS-REYMOND to calculate the electromotive force of a single plate of a column in the unexcited state,

which he finds may equal $\cdot 0000117$ of a DANIELL. No comparison is given between the current found to be present in the entire organ and that existing in the cut strip of tissue, from which it would seem that the different condition of the tissue in the two cases was considered as standing in no relation to the production of the current. My experiments show that, so far from this being the case with the tissues which I examined, there is a very marked difference between the organ-current shown by an entire organ and that shown by a cut strip. The galvanometer used in these experiments was a THOMSON of high resistance, made by ELLIOTT, which was brought to such a degree of sensibility that $\cdot 0001$ RAOULT gave, with a resistance of 10,000 ohms in the circuit besides that of the instrument (5332 ohms), a deflection of 230 scale. By means of the compensator (described by BURDON SANDERSON (22)) the organ-current could be "balanced," and the electromotive condition of the tissue thus measured. The balancing circuit was arranged in the manner described by the same writer, but a CALLAUD cell was used as the constant balancing battery, and the measurements were made in terms of this. The CALLAUD cell is a gravity battery used in telegraphy, and is a modification of THOMSON's gravity battery. As the copper is immersed in sulphate of copper, and the zinc in sulphate of zinc, its electromotive force is that of a RAOULT. The requisite determinations for the purpose having been made, the differences of potential are here given in terms of a RAOULT.

The amount and direction of the organ-current in the entire organ were ascertained in ten instances; the fish were killed by destruction of the brain, but were otherwise uninjured. The whole fish was therefore used for experiment, and was fixed with its ventral surface against a vertical board; this was perforated so as to allow the ventral surface of the organ to be reached by a leading-off electrode. The dorsal and ventral surfaces of the organ were now led off by kaolin cushions moistened with $\cdot 6$ per cent. saline, and connected with U-shaped non-polarisable electrodes. The points selected for leading off were opposite one another on the respective surface, and were situated in the middle of the organ. It is surprising, in the light of DU BOIS-REYMOND's statements, what contradictory results were thus obtained, as the following Table shows. The difference of potential existing between the dorsal and ventral electrodes is indicated in terms of the dorsal electrode. The sign +, therefore, signifies that the dorsal surface led off is galvanometrically positive to the ventral surface, and that a current passes through the organ columns from their ventral to their dorsal ends.

SMALL Torpedoes.—Six animals investigated.
(Dorsal surface as compared with ventral surface.)

| | | |
|-------------------|-------------------|-------------------|
| + $\cdot 002$ R; | + $\cdot 001$ R; | + $\cdot 0006$ R. |
| — $\cdot 0009$ R; | + $\cdot 0005$ R; | + $\cdot 0015$ R. |

MEDIUM-SIZED Torpedoes.—Four animals investigated.

| | | | |
|-------------------|-------------------|-------------------|-------------------|
| + $\cdot 0017$ R; | — $\cdot 0011$ R; | — $\cdot 0008$ R; | — $\cdot 0012$ R. |
|-------------------|-------------------|-------------------|-------------------|

The difference between the two contacts is thus seen to be very small, and in four cases out of ten was opposite in character. It was evident that nothing could be made out with certainty as to the state of matters in the entire animal. On the other hand, the blocks of tissue which were cut from these and other animals for rheotome experiments never showed any such discrepancy. In these the difference was always such that the dorsal end of the block was positive to the ventral end. It is needless to give examples of this now, for the experiments which will be given will afford convincing proof of the truth of this statement; but it may be stated that in 45 instances in which the organ-current of a cut strip or block of tissue was observed and noted there is no instance of the current being otherwise than +, and that in all cases the strength of the current far exceeded that which was observed in the case of the entire animal.

The difference observed very early in the work between the organ-current in the cut-out block of tissue, and that in the organ when *in situ*, suggested the view that the increased current found to be present in the former tissue was dependent upon the nature of the preparation—upon the fact that the piece of organ used was bounded by incisions. It was, therefore, determined to ascertain how far such incisions did affect the result. For this purpose the following experiment was carried out:—A medium-sized Torpedo, 18 centims. long by 12 wide, and 15 millims. thick at the median border of the organ, was fixed on a perforated vertical board; the brain had been previously destroyed, but the other parts of the fish remained intact. The skin covering the dorsal and ventral surfaces of the right organ was now led off by kaolin plugs, the leading-off points being situated in the middle of the length of the organ, and 5 millims. outside its median border; and care was taken that the one electrode should be exactly opposite the other. The dorsal surface was found to be negative to the ventral; the difference of potential was, however, quite inconsiderable, and amounted to — 001 RAOULT.

The organ, with its skin coverings, was now cut through a few millimetres in front of the leading-off electrodes. The direction of the cut was from without inwards and slightly backwards, and did not extend beyond the median border of the organ. On examining the tissue two or three minutes after the incision, the difference of potential was found to be the reverse of what it had been; the dorsal contact being now positive to the ventral, the difference amounted to + 0005 R.

A second cut was now made at right angles to the first cut, and thus nearly parallel with the long axis of the fish. It was external to the leading-off contacts, and extended through the length of the organ. The tissue now led off was, therefore, a long block bounded internally by the median border of the organ, anteriorly by the first cut, externally by the second cut, and posteriorly by the posterior boundary of the organ. The difference of potential was found to be still further increased after this incision, and amounted to + 0015 R.

The left organ of the same fish was now taken, and a large wedge-shaped block

cut from it. This was effected by two vertical incisions through the organ in the direction of its columns, extending from the median to the external border of the organ. The cuts were parallel, and were 12 millims. apart. The block of tissue was separated from the gills which lie close to the median border of the organ. It was thus a slice of organ the dorsal and ventral surfaces of which remained covered by skin. This preparation was placed upon a glass plate, resting with one of its cut surfaces in contact with the glass; the dorsal and ventral skin surfaces were now led off by kaolin cushions, and the difference found to be $+ \cdot 0002$ R. The slice was now reduced in thickness by cutting it into two thinner slices, the cut being parallel to the previous ones. One of these long slices, which resembled the previous one in all respects except that it was only 6 millims. in thickness, was now examined, and the difference found to be $+ \cdot 0007$ R. This slice was still further reduced by another cut, and now measured only 3 millims. in thickness. The skin still remained covering the ends of the columns, and when led off a marked difference of potential was observed. This difference declined slowly; it was compensated at intervals of one minute.

| | R. |
|--------------------------------------|------------------|
| 2 minutes after the cut difference = | $+ \cdot 0047$ |
| 3 " " " " | $= + \cdot 0043$ |
| 4 " " " " | $= + \cdot 0037$ |
| 5 " " " " | $= + \cdot 0032$ |
| 6 " " " " | $= + \cdot 0030$ |
| 7 " " " " | $= + \cdot 0027$ |
| 8 " " " " | $= + \cdot 0025$ |
| 15 " " " " | $= + \cdot 002$ |

It declined now very slowly, and another incision was therefore made. This was across the slice at right angles to the previous cuts. The experimental portion was therefore reduced to a small block of tissue, of the same thickness as before (3 millims.), but with a cut edge running parallel to its median border. The difference of the skin contacts was $+ \cdot 002$ R. before the cut, and after the cut it was found to have largely increased.

| | R. |
|-------------------------------------|------------------|
| 1 minute after the cut difference = | $+ \cdot 0051$ |
| 2 minutes " " " | $= + \cdot 0039$ |
| 3 " " " " | $= + \cdot 0031$ |
| 4 " " " " | $= + \cdot 0028$ |
| 5 " " " " | $= + \cdot 0025$ |
| 6 " " " " | $= + \cdot 0023$ |

This block was now divided in the direction of the columns, a small prism of organ being thus made 12 mm. in length in the direction of the columns, 5 mm. in breadth, and 3 mm. thick. Three minutes after its preparation it was examined, and the difference of the ends of the columns found to be $+ \cdot 0039$ R.

From the preceding experiments we are led to conclude that after cutting the organ an electromotive change occurs in the columns bordering on the cut, of such a character that the dorsal ends of the columns are positive to the ventral ends. The change is evidently that observed by DU BOIS-REYMOND in strips of tissue, and called by him the organ-current. It subsides rapidly at first, and then more and more slowly, and is in this respect analogous to the demarcation-current produced by injury in muscle and nerve.

In the latter tissues a prolonged electromotive change is produced when a portion of the tissue is injured by mechanical, thermal, or chemical means; this effect slowly subsides; it is increased when the tissue in the neighbourhood of the injury is warmed, diminished when that part is cooled. In both muscle and nerve the demarcation-current is the sum of changes produced by the injury in a number of electromotive elements, and its amount is therefore dependent upon the number of muscle fibres or nerves which are involved in this injury. The effective injury for its production in these tissues is thus a section which shall cut at right angles to their length as many fibres as possible.

Now, in the organ of the Torpedo the electromotive elements, plates, and nerves are disposed at right angles to the length of the framework which encloses them, and which constitutes a column.

A proceeding analogous to that of making a transverse section of the nerve fibres of a nerve trunk is thus carried out upon the organ columns when an incision is made through the organ parallel to the columns. Such an incision must cut at right angles to their length a large number of plates and nerves; this should be followed by an electromotive change which expresses the sum of the local changes occurring in the immediate neighbourhood of the cut.

This is what I conceive to be the meaning of the organ-current. Like all "demarcation" currents, it is the expression of a protracted state of excitation of the still living tissue in the neighbourhood of the injury. The corresponding electromotive change must manifest itself as all the excitatory phenomena of the Torpedo manifest themselves, namely, as a current passing through the column from the ventral to the dorsal surface.

In support of this assertion we will now pass to experiments as to the effect of local warming of an incised surface.

From a small Torpedo 13 centims. long by 9 centims. wide a slice of organ was cut, which was then divided up into three blocks. Each block comprised the whole length of the columns which remained covered by skin at their dorsal and ventral ends. Each was 2 mms. wide and 3 mms. thick, and thus consisted of several unimpaired columns surrounded by columns which had been cut through parallel to their length. These cylinders of tissue were now examined, the skin ends being led off by kaolin cushions. The first cylinder, examined five minutes after its preparation, showed a difference of potential = + 0045 R., subsiding rapidly to + 0034 R., and then more slowly.

A hot iron wire was now brought within a few millimetres of one of the cut sides of the preparation; the difference immediately began to rise, as was shown on the galvanometer by a slow deflection amounting to more than 200 scale. On withdrawing the wire, the difference subsided; on approaching it, it rose again. The second cylindrical block was examined in the same way half-an-hour after its preparation. The difference was found to be $+ \cdot 0017$ R. One of the cut sides was now warmed as before, and the difference rose; when it had attained its maximum of rise it was compensated and found to be $+ \cdot 002$ R. On withdrawing the hot iron wire it fell again, but on again bringing the hot wire near the cut surface it rose to $+ \cdot 0022$ R.

Similar results were obtained with the third cylinder, and could always be brought about in such preparations. In this respect, then, the organ-current resembles the demarcation-current of muscle and nerve.

One of the most efficient methods for the production of a demarcation-current in the last-named tissues is that of making what is termed a "thermal section." This method has the advantage of injuring the tissue locally without altering its dimensions to any considerable extent. It should be possible to produce similar thermal sections in the case of the Torpedo. From what has been said as to the structure of the electrical organ it is evident that the appropriate thermal section would be obtained if a block of tissue could be prepared which should consist of a few unimpaired columns surrounded by other columns partially in a state analogous to heat rigor. This was effected by the following simple plan:—A cubical block of tissue, consisting of several columns with their ends covered by skin, was held by its dorsal skin in the forceps, and then plunged in hot water so as to be wholly immersed for two seconds. On examining the block, the sides were seen to be opaque-looking. They were undoubtedly destroyed, but in the interior the substance of the columns appeared to be unimpaired. In such a block a thermal section has therefore been made on every plate and nerve branch within a millimetre or so of the surface. The resulting difference should therefore be very large, and investigation showed that this was the case.

Three strips were cut from the organ of a large Torpedo. The first was cut from the median edge of the organ. The length of its columns was 55 mms., and the strip was 3 mms. in thickness and in width. It was immersed in hot water for two seconds, and then examined by leading off the skin covering the ends of the columns. The difference was found to be very large, amounting to as much as $+ \cdot 052$ R.

The second strip was cut from the organ midway between its median and outer borders. Its length (23 mms.) was less than the previous one, owing to the columns being shorter at this point, but in other respects it resembled the first strip. On examining the difference two minutes after immersion it was found to be $+ \cdot 0336$ R.

A third strip was cut from the outer border of the organ, where the columns measured only 12 mms. in length. It was otherwise of the same dimensions as the

preceding ones. It was immersed in hot water for two seconds, and the difference two minutes afterwards was found to be $+ \cdot 0192$ R.

This experiment left no doubt as to the possibility of producing effects comparable with those of thermal section by an analogous mode of injury, and it is to be remarked that the electromotive change produced is the greater, the longer the columnar strip. This is due to the fact that the number of electromotive elements operated upon is proportional to the length of the strip.

Another experiment was made upon a strip cut from a medium-sized Torpedo, and measuring 25 mms. in length and 3 mms. in thickness and breadth. This strip was kept in the cold for one hour and then examined; the difference was found to be $+ \cdot 0021$ R. It was now immersed for a few seconds in hot water and re-examined, when the difference had increased to as much as $+ \cdot 0226$ R.

Since the effect is produced by injury of the plates, which injury causes an electromotive change in the plates such that the ventral aspect of the plate is negative to the dorsal, it must follow that, whether the injury extends along a whole column or only along a small portion, the effect is always of similar direction and character.

The electromotive plates respond to the local excitation of injury as they do to nerve excitation, but the response in the former case is comparatively feeble, and is very prolonged. Wherever the injury may be, the effect must, therefore, be that the dorsal surface of the column is positive to the ventral surface. If, for instance, the dorsal half of a column be immersed in hot water, the effect must be of similar character to that produced by total immersion, and the same must be the case if the ventral half be immersed. The difference between partial and complete immersion is merely a difference in the amount, not in the direction of the effect produced, the amount being proportional to the number of electromotive elements affected.

Three strips were cut from the organ of a large Torpedo, each measuring 20 mms. in length of column, and 3 mms. in thickness and width. The first strip, when examined half-an-hour after its preparation, showed a difference of $+ \cdot 0091$ R. The dorsal half of the strip was immersed in hot water for two seconds, and the difference rose to $+ \cdot 0209$ R. The second strip showed a difference of $+ \cdot 0084$ R.; the ventral half of this was immersed, and the difference rose to $+ \cdot 0254$ R. The third strip was wholly immersed, and the difference amounted to $\cdot 0340$ R.

In another experiment upon a strip cut from a medium-sized Torpedo, the columns of which measured 25 mms. in length, the strip was found to show a difference, an hour and a half after its preparation, amounting to $+ \cdot 0007$ R. The dorsal fourth of the strip was immersed for two seconds, and the difference rose to $+ \cdot 003$ R.; as soon as possible the ventral fourth of the same strip was immersed, and the difference rose to $+ \cdot 0065$ R.; the whole strip was now immersed, and the difference rose to $+ \cdot 0228$ R.

These results justify the statements which preceded them, and show that, whatever the part injured, the effect is the same, the organ-current being *always similarly directed through the columns, namely, from the ventral to the dorsal surface.*

There is a possible source of fallacy connected with the nature of the preparation. The skin may have electromotive properties, and electromotive changes may follow its injury. The subject has been investigated by DU BOIS-REYMOND, but without any decisive results (23). It would appear, however, that if the skin is electromotive it is only feebly so, and any change from the skin must, in blocks of organ, be swamped by the larger organ-current. Experiments were, however, made upon the subject at Arcachon which showed that in the injured skin the surface becomes negative to the deeper uninjured parts. We should, therefore, expect that injury of the dorsal skin in the case of an immersed block of tissue would cause an electromotive change opposing that of the organ, whilst similar injury of the ventral skin would cause a change favouring that of the organ. If this be so, then in the case of a strip of organ with skin remaining on its dorsal and ventral surfaces, which has been totally immersed in hot water, the total electromotive change is that of the plate's organ-current *plus* that of the ventral skin *minus* that of the dorsal skin; the skin effects, one opposing and the other favouring the total organ effect, thus balance each other. But, if a strip be cut and the organ-current allowed to subside, injury to the dorsal skin only should be followed by sinking of the difference, injury of the ventral skin only by rise of the difference. The following experiments justify this view. In a strip 25 mms. long and 3 mms. in width and breadth, examined one hour after preparation, the difference between dorsal and ventral surfaces was $+ \cdot 0021$ R. The strip was now held up by its ventral skin, and the dorsal skin allowed to touch a hot surface. On examining the difference, it had diminished to $- \cdot 0008$ R. The same strip was now held up by its dorsal skin, and the ventral skin was allowed to touch a hot surface: the difference, when examined, had increased to $+ \cdot 002$ R. Finally the whole strip was immersed in hot water for two seconds, when the difference was found to be $+ \cdot 0226$ R.

In another strip, cut from a small Torpedo, and measuring 13 millims. in length, the difference 15 minutes after preparation was found to be $+ \cdot 002$ R. The dorsal skin was now carefully removed, and the contacts were made upon the ventral skin and the dorsal ends of the columns respectively. The difference had diminished to $+ \cdot 0015$ R. The ventral skin was carefully cut off, the contacts now being made upon the uncovered ends of the columns, and the difference found to be $+ \cdot 0027$ R. In the operation of removing the skin it is difficult to avoid injuring the organ columns; such injury would always produce a $+$ effect, which would have to be taken into consideration.

It seems, then, that in the injured skin the surface becomes negative to the uninjured deeper parts. It is, therefore, not improbable that the very small currents observed in entire Torpedoes are, to a large extent, skin currents, these being produced by the injuries which the skin must receive in the process of capture, handling, &c.; but the question is one which demands further investigation. It is obvious that it does not affect the present inquiry.

An "organ-current" may be reproduced in a strip of tissue 24 hours after the preparation of the strip. Thus a block of organ was cut from a medium-sized

Torpedo: the difference amounted to $+0.0062$ R.; it was left until the next day, being kept in the cold and surrounded with other fragments of organ to keep it moist.

When examined after 24 hours, the difference was found to be very small—indeed hardly perceptible. It was now wholly immersed for two seconds in hot water, and the difference was examined. It amounted to $+0.0195$ R.; in three minutes it sank to $+0.013$ R., then to $+0.008$ R., $+0.006$ R., $+0.005$ R., $+0.0043$ R., $+0.0036$ R., &c., the difference being compensated every three minutes.

It has been observed that the difference following injury is of such a character that it declines at first rapidly and then more slowly.

Experiments were made to ascertain the rate of this decline. For this purpose the effect was recompensated at regular intervals; the results of three series are now given with strips led off by their dorsal and ventral skin ends:—

| Time after preparation. | Cut strip 40 millims. long. | Cut strip 15 millims, immersed for 2 minutes in hot water. | Cut strip 15 millims, immersed for 2 minutes in hot water. |
|-------------------------|-----------------------------|--|--|
| minutes. | | | |
| 2 | diff. $+0.0275$ R. | $+0.0255$ R. | $+0.0226$ R. |
| 3 | $+0.0230$ | $+0.0180$ | $+0.0175$ |
| 4 | $+0.0205$ | $+0.0135$ | $+0.0145$ |
| 5 | $+0.0180$ | $+0.0125$ | $+0.0135$ |
| 6 | $+0.0160$ | $+0.0117$ | $+0.0112$ |
| 7 | $+0.0140$ | $+0.0110$ | $+0.0106$ |
| 8 | $+0.0126$ | $+0.0102$ | $+0.0100$ |
| 9 | $+0.0117$ | $+0.0095$ | .. |
| 10 | $+0.0110$ | $+0.0090$ | .. |
| 11 | $+0.0100$ | $+0.0086$ | .. |
| 12 | $+0.0092$ | $+0.0083$ | .. |
| 13 | $+0.0087$ | $+0.0081$ | .. |
| 14 | $+0.0082$ | $+0.0078$ | .. |
| 15 | $+0.0076$ | .. | .. |
| 16 | $+0.0071$ | .. | .. |
| 17 | $+0.0068$ | .. | .. |
| 18 | $+0.0065$ | .. | .. |
| 19 | $+0.0062$ | .. | .. |
| 20 | $+0.0059$ | .. | .. |
| 21 | $+0.0056$ | .. | .. |
| 22 | $+0.0053$ | .. | .. |
| 23 | $+0.0050$ | .. | .. |
| 24 | $+0.0048$ | .. | .. |
| 25 | $+0.0046$ | .. | .. |
| 26 | $+0.0045$ | .. | .. |
| 27 | $+0.0044$ | .. | .. |

The effect is seen to decline rapidly at first and then slowly. This is a very characteristic feature of the organ-current, and, apart from its obvious resemblance to the demarcation-current observed in cardiac tissue, is strongly suggestive of the excitatory nature of the whole phenomenon. A point of great interest in this connection is the influence on the rate of the decline of a nerve-organ response. The experiments which were made on this subject were not, however, satisfactory,

owing to the lack of suitable methods; they only showed that the decline was more rapid when the organ was excited.

The facts relating to the organ-current may now be summed up as follows :—

The difference between the dorsal and ventral surface of the organ is very small in the uninjured organ when examined *in situ*, and under these circumstances is often of opposite sign in different animals.

When a portion of cut tissue is examined an electromotive difference is always found to be present, and is such that the dorsal surface of the columns is positive to the ventral.

This difference subsides at first rapidly, and then so slowly that it may be observed one or two hours after the preparation has been made.

The difference is produced by incisions which cut through the columns in the direction of their length, thus cutting the electromotive elements at right angles to their length.

The most effective method for its production is the thermal section of the external columns contained in a strip of the tissue.

The amount and not the direction of the effect is affected by partial injury of a column, whether this be dorsal or ventral.

The organ-current is thus analogous to the demarcation-current of muscle and nerve, and is believed to correspond to a prolonged local excitation of the vital electromotive elements in the immediate neighbourhood of the injury.

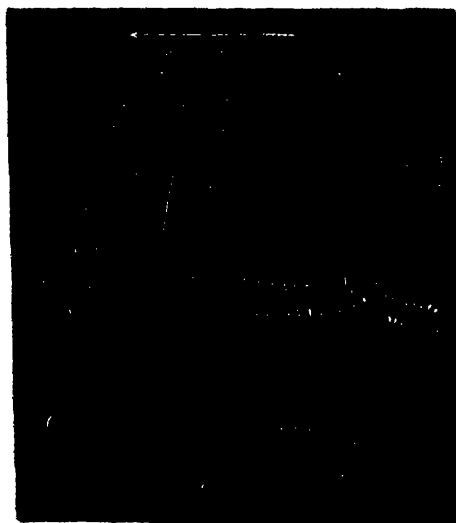
II. *Experiments relating to the Time Relations of the Excitatory Change in the Organ following Excitation of its Nerve.*

The method used in these experiments was that rendered familiar by its employment in experiments of a similar kind upon other tissues, particularly cardiac tissue. This consists in the use of a rheotome which shall connect the galvanometer with the tissue under investigation during a known period after excitation of the tissue. In the experiments of MAREY upon the Torpedo (24) a fast-travelling plate, such as that of the pendulum or spring myograph of DU BOIS-REYMOND, was used. The movement of the plate was arranged to ensure both the passage of an induction shock at a given point in its course and a definite closure (for $\frac{1}{1000}$ "") of the circuit which connected the organ of the Torpedo with the "physiological galvanoscope," this being the nerve muscle preparation of the frog—the movements of which were recorded on the travelling plate. This method had been used by myself in ascertaining the time relations of the response of the *Malapterurus* to excitation of its skin (25). The information which this method gives is, however, of relatively little value, since it is obtained in terms which bear no sort of relation to those applicable to electrical phenomena in general, such as the deflections of a galvanometer needle of known sensibility, &c. To obtain such information as this latter was the object of the following experiments.

For this purpose I had taken with me a spring myograph, constructed on the plan of DU BOIS-REYMOND, but furnished with three special keys to fit it for use as a rheotome.

The passage of the traveller opened these keys in succession; they could be adjusted with great accuracy at any distance one from the other. The three keys may be distinguished as K_1 , K_2 , K_3 . These were arranged so that the break of K_1 opened the primary circuit of an induction apparatus; that of K_2 a circuit which, when closed, short-circuited the galvanometer; and K_3 the galvanometer circuit itself. The galvanometer was the same as in the previous experiments, and was brought to a degree of sensibility that, with a resistance of 10,000 ohms, besides that of the instrument, .0001 R. gave a deflection of 230 scale. It was often necessary to allow only $\frac{1}{10}$, $\frac{1}{100}$, or $\frac{1}{1000}$ of the total current in the circuit to pass through the instrument. This was effected by the shunt provided with the instrument, and the terms $G. \frac{1}{10}$, $G. \frac{1}{100}$, $G. \frac{1}{1000}$, express this in all the experimental data which follow. Since the use of the shunt diminishes the total resistance in the circuit, a resistance of 10,000 ohms was introduced in order to ensure the complete short-circuiting of the galvanometer by K_2 when the shunt was used. The whole arrangement is given in fig. 2. The traveller of the rheotome moved with sufficient rapidity to allow an accurate adjustment of the keys to within $\frac{1}{1000}$ " of each other; the movement was timed by recording on the plate of the traveller the vibrations of a tuning-fork vibrating 100 times in a second.

Fig. 2.



The induction apparatus used for excitation was constructed and graduated under the kind direction of Professor KRONECKER, of Berne.

The first experiments were made with the view of testing the arrangements, as it was necessary to see how far the organ response could be investigated in this manner. For this purpose a small Torpedo 13 cm. \times 9 cm. was selected, and, the brain having been destroyed, the second, third, and fourth electrical nerves were exposed and freed

as far as the gills. The organ was left untouched, and the animal fixed on a perforated vertical board. Through the perforation the ventral surface of the organ was led off by one non-polarisable electrode, the other being placed upon a corresponding point on the dorsal skin covering the organ. A pair of fine platinum electrodes were placed under the second nerve.

There was a slight electromotive difference between the dorsal and ventral contacts, amounting to $+ \cdot 002$. The nerve was now excited by the break of the current of two Grove cells placed in the primary circuit of the induction apparatus. It was noticed that a considerable strength of induction current had to be used before an organ response could be obtained. This point has been observed and commented upon by DU BOIS-REYMOND (26). With the secondary coil at 5 cm. the galvanometric effect obtained by excitation was $= + 750$ scale G. $\frac{1}{10}$; the sign $+$ signifying that a current passed through the galvanometer directed from the dorsal to the ventral leading-off electrode.

A preliminary rheotome experiment was now made, the short-circuiting key K_2 being fixed at $\cdot 01''$ after the exciting key K_1 , whilst K_3 was moved so as to lengthen the duration of the galvanometer closure.

| Time of closure of galvanometer, the moment of excitation being zero } | $\cdot 01'' - \cdot 014''$ | $\cdot 01'' - \cdot 015''$ | $\cdot 01'' - \cdot 016''$ | $\cdot 01'' - \cdot 0165''$ | $\cdot 01'' - \cdot 0225''$ | $\cdot 01'' - \cdot 0275''$ |
|---|----------------------------|----------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Deflections observed in gal- vanometer. (Where more than one reading is given, it indicates another experi- ment under same condi- tions.) | 0 0 0 | + 65 + 72 + 65 | + 240 .. | + 550 .. | + 500 G. $\frac{1}{10}$ | + 650 G. $\frac{1}{10}$ |

This showed that in this preparation the electromotive response, as indicated by the galvanometer, commenced at $\frac{1}{1000}''$ after excitation of the nerve, and was fully developed by $\frac{2}{1000}''$ after excitation.

The rapid development of the response is also shown in the next experiment, in which the organ of the other side of the same animal was led off, and all the electrical nerve trunks excited. Here, with a galvanometric closure of from $\cdot 01 - \cdot 014$, no effect occurred after excitation; with $\cdot 01 - \cdot 0155$, an effect of $+ 195$ scale; with $\cdot 01 - \cdot 0165$ a large effect shot the needle off the scale ($+\infty$).

On examining this same preparation half-an-hour or so later, the time relations of the effect were found to have changed thus:—

| Galvanometer closure . . | $\cdot 01'' - \cdot 012''$ | $\cdot 01'' - \cdot 0125''$ | $\cdot 01'' - \cdot 014''$ | $\cdot 01'' - \cdot 015''$ |
|--------------------------|----------------------------|-----------------------------|----------------------------|----------------------------|
| Deflections | 0 0 | + 55 | + 165 | $+\infty$ |

This change was subsequently found to be due to the high temperature of the room (20° C.) affecting that of the preparation. The experiment showed that it was possible to obtain with the instrument very sharp differences, with small differences in time, amounting to only $\frac{1}{1000}$ ".

It was now necessary to see if the same would hold good of a cut piece of organ with nerve attached, a nerve-organ preparation. A large fish, 48 cm. X 30 cm., was selected, and the brain destroyed. The largest electrical nerve (the 2nd) was then prepared from its exit from the skull to its entry between the gill clefts. A great sector of organ, comprising the distribution of this nerve, was then made by two clean cuts with a razor through the skin and the organ in the direction of the columns, the cuts reaching from the median to the outer border of the organ. The mass of separated organ measured 40 millims. in thickness at its median edge; this, therefore, represents the length of the columns at this point. It was fixed on a vertical board, and led off by cushions of saline kaolin similarly placed on its dorsal and ventral surfaces. The fish had been caught the night before the experiment, and the temperature of the organ was still very low, only 5° C.

The block of tissue gave the usual "organ-current," = + .0052 R., which subsided slowly during the experiment. The nerve was excited 6 centims. from its point of entry into the organ by the break of two Groves in the primary coil.

No response to the passage of the induction shock was observed until the secondary coil was pushed up to 8 cm., when a response of + 105 G. $\frac{1}{10}$ occurred. On pushing the coil to 5 cm., the response increased to + 350 G. $\frac{1}{10}$.

| | | | | |
|----------------------|------------|------------------------|-------------------------|-------------------------|
| Closure | ·015"-·02" | ·02"-·0225" | ·0225"-·0275" | ·0275"-·0335" |
| Deflection | 0 0 | + 70 G. $\frac{1}{10}$ | + 150 G. $\frac{1}{10}$ | + 260 G. $\frac{1}{10}$ |

| | | | |
|----------------------|-------------------------|------------------------|-----------------------|
| Closure | ·0335"-·0385" | ·0385"-·0435" | ·0435"-·05" |
| Deflection | + 220 G. $\frac{1}{10}$ | + 15 G. $\frac{1}{10}$ | + 3 G. $\frac{1}{10}$ |

The temperature of the room subsequently affected the time relations of the response, as one hour afterwards the response was found to begin earlier.

| | | | |
|--------------------------|------------|--------------|--------------|
| Galvanometer closure . . | ·01"-·015" | ·015"-·0165" | ·0165"-·018" |
| Deflection | 0 | + 15 | + 150 |

The interval between nerve excitation and the commencement of the response was much longer than had been anticipated.

In the particular experiment just given, about '006" would be occupied by transmission along the nerve trunk, leaving '01" still unaccounted for. The influence of temperature seemed to indicate that the delay was connected with the low temperature of the fish. To obviate this, the Torpedoes were now kept in water at 12° C. It was now deemed necessary to repeat JOLYET's experiments on transmission.

Experiments on Transmission in the Electrical Nerves.

Three distinct sets of experiments were made on this subject.

A medium-sized vigorous Torpedo, 26 cm. \times 17 cm., which had been kept for some days in water at 12° C., and gave smart shocks to the hand, was killed by punching the brain. The second electrical nerve was now prepared to its point of entry into the organ, and a wedge of this tissue was then cut out corresponding to the distribution of the nerve. The preparation was placed upon a glass plate, and the dorsal and ventral skin led off by cushions of kaolin. The nerve hung free in air, supported on the platinum exciting electrodes. It had been observed in previous experiments that the nerve was much less excitable in that part of its course which was in close proximity to the organ; the electrodes could not, therefore, be shifted with safety any great distance along the nerve.

Organ-current = + '0042 R.

Induction coil without core in primary coil.

Secondary coil at 0. Two Groves in primary circuit.

EXCITATION 25 millims. from organ.

| | | | | |
|----------------|-----------|------------|------------|--------------------------|
| Closure . . . | '008"-01" | '008"-011" | '008"-012" | '008"-on |
| Deflection . . | 0 0 | + 170 | + ∞ | + 255 G. $\frac{1}{100}$ |

EXCITATION 12 millims from organ.

| | | | | | |
|--------------|------------|-----------|-----------|------------|-------------------------|
| Closure . . | '007"-008" | 008"-009" | '008"-01" | '008"-011" | '008"-on |
| Deflection . | 0 | + 55 | + 660 | + ∞ | + 85 G. $\frac{1}{100}$ |

EXCITATION 25 millims. from organ.

| | | | |
|------------------|-----------|------------|------------|
| Closure | '008"-01" | '008"-011" | '008"-012" |
| Deflection . . . | 0 | + 220 | + ∞ |

EXCITATION 12 millims. from organ.

| | | | |
|--------------------|-------------|-------------|------------|
| Closure | ·007"—·008" | ·008"—·009" | ·009"—·01" |
| Deflection | 0 | + 60 | + 650 |

Another preparation was made from the organ of the opposite side of the same fish, of same character and dimensions. It was examined four hours after the first preparation had been begun, and gave the following results:—

EXCITATION 25 millims. from organ.

| | | | |
|--------------------|------------|------------|-------------|
| Closure | ·008"—·01" | ·01"—·012" | ·01"—·0125" |
| Deflection | 0 | 0 0 | + 255 |

EXCITATION 12 millims. from organ.

| | | |
|--------------------|------------|-------------|
| Closure | ·008"—·01" | ·008"—·011" |
| Deflection | + 20 | + 195 |

In the fresh preparation the commencement of the electromotive change in the organ occurs $\frac{2}{1000}$ " earlier when the excitation of the nerve is 13 millims. nearer the organ. The excitatory process in the nerve thus travels at a rate of 6·5 metres per second. As the total electromotive change is less when the nerve is excited at the point nearer the organ, the actual commencement of the response may be too small to be observed; if there be any such error, it would imply that the above transmission rate is too rapid.

The second experiment, made four hours later, gives a transmission rate of 5·2 metres per second.

In another experiment on a nerve-organ preparation, made from the organ of a large Torpedo, the organ was kept at 5° C., whilst the nerve was kept at the temperature of room, 12° C.

EXCITATION of nerve 23 millims. from organ.

| | | | | |
|--------------------|-------------|-------------|-------------|------------|
| Closure | ·016"—·017" | ·017"—·018" | ·018"—·019" | ·019"—·02" |
| Deflection | 0 0 | + 90 | + 340 | + 480 |

EXCITATION of nerve 43 millims. from organ.

| | | | | | | |
|--------------------|-------------|-------------|-------------|------------|--------------|-------------|
| Closure | ·016"-·017" | ·017"-·018" | ·018"-·019" | ·019"-·02" | ·02"-·021" | ·021"-·022" |
| Deflection | 0 | 0 | 0 | 0 | + 50 + 65 | + 310 |

This gives $\frac{3}{1000}$ " as the time of transmission along 20 millims. of nerve, a rate of 6·6 metres per second.

A third experiment was made upon a nerve-organ preparation of a large vigorous Torpedo, measuring 52 cm. X 37 cm. The fish had been kept at 12° C., and the preparation differed from the preceding, since it consisted of *one* long uninjured column surrounded by cut columns. This column measured 6 centims. in length, and was 5 millims. in cross-section. The nerve entering it was carefully dissected out, and measured 8 centims. in length. The very large organ-current was allowed to subside, the experiment being deferred for an hour or more. A small patch of skin remained on each end of the column, and was led off in the usual way: O.C. = + ·0031 R. Induction apparatus as in preceding experiment.

EXCITATION of nerve 65 millims. from organ.

| | | | | | | |
|----------------------|------------|------------|-------------|-------------|------------|------------|
| Closure | ·01"-·012" | ·01"-·014" | ·014"-·016" | ·016"-·018" | ·018"-·02" | ·02"-·022" |
| Deflection | 0 | 0 | 0 | + 70 | + 140 | + ∞ |

EXCITATION of nerve 25 millims. from organ.

| | | | |
|----------------------|------------|-------------|-------------|
| Closure | ·01"-·012" | ·011"-·013" | ·012"-·014" |
| Deflection | 0 | + 120 | + ∞ |

EXCITATION of nerve 65 millims. from organ.

| | | | |
|----------------------|-------------|-------------|-------------|
| Closure | ·015"-·016" | ·016"-·017" | ·017"-·018" |
| Deflection | 0 | 0 | + 70 |

EXCITATION of nerve 25 millims. from organ.

| | | |
|-----------------|------------|-------------|
| Closure | ·011"—·012 | ·011"—·013" |
| Deflection . . | + 20 | + 220 |

The mean of these readings gives ·0055" as the time occupied by transmission down 40 centims. of nerve—that is to say, the nervous impulse travels down the trunk of the electrical nerve at the rate of 7·3 metres per second.

This is practically the same result as that obtained by JOLYET (27) with vigorous summer fish, and gives substantial confirmation of his statements as to the slow rate of transmission in the electrical nerves of the Torpedo.

Experiments upon the Time of Delay.

When we have made the necessary deduction for transmission time, we find that an interval between excitation of nerve and response of organ still remains. In the particular experiments just given this amounts to ·006". This is the "temps perdu," or time of delay, and we now pass to experiments having reference to the influence upon the length of this period of varying conditions of the organ itself. The principal conditions which affect the response in this respect—that is to say, which affect its commencement—may be considered under three heads:—(1) The temperature of the organ; (2) the strength of the nerve stimulus which evokes the response; (3) the vigour of the particular organ under investigation, and thus the vigour and size of the fish.

(1.) *Influence of temperature.*—A ready means of altering the temperature of the organ was secured by the use of a long water-box, through which water at different temperatures could be run, and which supported on its surface the organ preparation. To prevent any galvanometric disturbance through the metal of the box, the metal was covered with thin paper soaked in melted paraffin.

A nerve-organ preparation was made from a large Torpedo, 50 cm. × 34 cm., and placed upon the tubes in such a way that, whilst the organ rested upon the warming apparatus, the nerve hung free in air. Rheotome observations were made every five minutes, so as to allow time for shifting the keys, it being necessary to catch the beginning of the response. The organ was at 5° C. at the commencement; it was warmed to 20° C. by allowing water at 45° to flow through the apparatus, and when the quickening-up of the response had ceased it was re-cooled. The result is given in the following Table. In the first experiment the nerve was excited 45 millims. from the organ, so that $\frac{1}{1000}$ " must be deducted from the interval as transmission time. With this deduction the time of delay is only $\frac{5}{1000}$ " with an organ at 20° C., whereas it is $\frac{12}{1000}$ " to $\frac{14}{1000}$ " when the same organ is at 5° C.

In Experiment 2 the time of delay is to be reduced to $\frac{4}{1000}$ " at a temperature of 20° C., from $\frac{14}{1000}$ " at a temperature of 5° C., the transmission time being ·0065".

(2.) *Effect of strength of stimulus.*—In all the preceding experiments a stimulus had always been used of such strength as to procure a large response of the organ. The importance of procuring a maximal contraction in the investigation of similar phenomena in muscle is well known, but in endeavouring to obtain a maximal response of the electric organ to stimulation of its nerve we are confronted with a difficulty which is not present in the case of muscle. The range of stimuli which call forth minimal effects in the latter is very limited; in the nerve-organ preparation, on the other hand, it is large. The organ is a better interpreter than the muscle of the change produced in a nerve by the passage of an induction current. A far stronger stimulus is necessary to excite the nerve-organ than the nerve-muscle preparation; but, once this strength has been reached, the nerve-organ preparation responds more and more fully, the stronger the stimulus, until, with a damaging strength of current, the nerve itself is injured. The following experiment is selected from several to illustrate this.

A nerve-organ preparation was made from the organ of a large Torpedo, 42 cm. \times 29 cm. The nerve was excited by the break of K_1 , and K_2 was so placed as to be broken '005" later; the third key, K_3 , was not used. The point of excitation was 20 millims. from the organ.

| Primary circuit with 2 Groves. | | | | Coil with core | Effect. |
|--------------------------------|---------|-------|--------|---------------------|------------|
| Secondary coil, 20 | centims | (150) | . . . | G. $\frac{1}{10}$ | 0 |
| " | 17 | " | (300) | G. $\frac{1}{10}$ | + 30 |
| " | 16 | " | (400) | G. $\frac{1}{10}$ | + 700 |
| " | 15 | " | (700) | G. $\frac{1}{10}$ | + ∞ |
| " | 17 | " | (300) | G. $\frac{1}{10}$ | + 15 |
| " | 16.5 | " | (350) | G. $\frac{1}{10}$ | + 45 |
| " | 16 | " | (400) | G. $\frac{1}{10}$ | + 650 |
| " | 15 | " | (700) | G. $\frac{1}{10}$ | + 110 |
| " | 12.5 | " | (2000) | G. $\frac{1}{100}$ | + ∞ |
| " | 12.5 | " | (2000) | G. $\frac{1}{1000}$ | + 90 |

(The numbers in brackets denote the relative strengths of the induction shocks.)

After an interval the strength of stimulus was still further increased.

| Primary circuit with 2 Groves. | | | | Coil with core | Effect. |
|--------------------------------|-----|--------|--------|--------------------|---------|
| Secondary coil, 12.5 centims. | | (2000) | . . | G. $\frac{1}{100}$ | + 365 |
| " | 10 | " | (4500) | G. $\frac{1}{100}$ | + 670 |
| " | 7.5 | " | (7000) | G. $\frac{1}{100}$ | + 620 |

With the secondary coil varied from 17 to 10 centims.—that is to say, with relative strengths of shock from 300 to 4500—the organ response became fuller, the stronger the stimulus. In the case of a large Torpedo the nerve bundles are of great thickness; the second nerve in the above experiment measured 5 millims. in diameter.

It is possible that the weaker induction current excites only the nerves upon the surface of the trunk; as the strength of the stimulus increases, more nerve fibres are traversed by currents of sufficient intensity to excite them. That this is not, however, all may be inferred from the existence of similar phenomena in the case of small newly-born Torpedoes, when the nerve bundles are small. Thus the minimal response of the organ to the excitation of the second nerve by the induction shock caused by the break of 4 Groves in the primary circuit occurred when the secondary coil stood at 24 centims., and the effect amounted to + 220 scale (Galv. without shunt); whereas the fullest response was only obtained when the secondary coil stood at 10 centims., and amounted to + 170 scale, G. $\frac{1}{100}$.

As in the case of muscle, so here we find that the magnitude of the response affects the period of delay. Thus in a small Torpedo the nerve was excited 10 millims. from the organ. The rheotome was used as in the temperature experiment, the galvanometer "window" (*i.e.*, interval between K_2 and K_3) being moved along so as to catch the commencement of the response.

| Galvanometer closure. | | | | '016"-018" | '018"-02" | '02"-022" | '022"-025" |
|-----------------------------|----|----------|----------|------------|-----------|------------|------------|
| Position of secondary coil, | 20 | centims. | (150) | .. | 0 | 0 | + 10 |
| " | " | 17.5 | " (320) | .. | 0 | + 110 | |
| " | " | 12.5 | " (2000) | .. | + 15 | + ∞ | |
| " | " | 10 | " (4500) | + 5 | + 200 | | |
| " | " | 5 | " (9500) | + 5 | | | |

The period of delay is here increased by '004" when the response is minimal.

(3.) *Influence of vigour and size of animal.*—The influence of the state of the fish is an obvious one: the organ responds more quickly, the more active and vigorous the fish used. It is needless to illustrate this, but a more interesting rider to the above is the influence of the size of the fish. In contrasting the results obtained from different fish, all in an equally good state, but of different size, it was found that the period of delay was always shorter, the larger the fish. Thus in four selected fish, all of which were kept at 12° C. for some days, all active and vigorous, giving sensible shocks to the hand, and in all of which the organ, as is shown below, responded very fully to nerve excitation, the following results were obtained.

a. Nerve-organ preparation from large Torpedo, 42 cm. \times 29 cm. Total response to nerve excitation with Galv. $\frac{1}{100}$ = + 650. The response commenced '007" to '008" after excitation of the nerve. The nerve was excited 20 millims. from organ, and, deducting '003" for transmission time, the period of delay is '004".

b. Nerve-organ preparation of medium-sized Torpedo, 26 cm. \times 17 cm. Total response with Galv. $\frac{1}{100}$ = + 400. The response commenced '008" to '009" after excitation of nerve. The nerve was excited 13 millims. from organ, and, deducting '002" for transmission time, the period of delay is '006".

c. Nerve-organ preparation of Torpedo 19 cm. \times 12 cm. Total response, Galv. $\frac{1}{100} = +50$. The response commenced '009" after excitation. The transmission time was '002", and the period of delay '007".

d. Nerve-organ preparation from Torpedo born in laboratory five days before; active, giving distinct shocks to the hand; length 12 centims., breadth 8 centims. Total response, G. $\frac{1}{100} = +80$. The response commenced '012" after excitation, and, the transmission time being at most '002", the period of delay is '01".

The increased time of delay shown by the organ of the smaller Torpedoes might be explained as simply a case of the preceding results, which showed that, the smaller the total response, the later its apparent commencement; but a comparison of Cases c. and d. shows that such an explanation is insufficient, since the response in d. is larger than that in c., yet it begins later.

These experiments upon the "time of delay" show the striking resemblance between the activity of the organ and that of muscle, nerve, &c. Experiments made eighteen months ago at Oxford, not yet published, upon the electromotive phenomena of skeletal Frog's muscle by the repeating rheotome method, showed that there is such a period of delay between the excitation of the nerve and the commencement of the electromotive change in the responding muscle. This period of delay was found to be about '004", but could be shortened by warmth and prolonged by cold. In the case of the cardiac tissue of the Frog the delay between direct excitation of the tissue and the appearance of an appreciable electromotive change under the proximal leading-off electrode varied with the distance between the exciting and leading-off electrode. When this distance was made very small the time was shortened to '03" (28). A longer interval still is found in the case of the Tortoise heart (29).

In the *Dionæa* a period of '05" intervenes between the excitation and the first appreciable electromotive change (30).

On the other hand, the *Malapterurus* discharge can be appreciated very soon after excitation of the organ itself; the time of delay here was with appropriate leading-off (31) shortened to '002".

The recognition of this delay is equivalent to the admission that existing methods of observation are inadequate, for it implies that with the best known methods of investigation there is an interval of time left unoccupied by any of the known phenomena of functional activity. The existence of a real time of rest in the molecular processes of the excitatory state, when these processes pass from nerve-trunks into nerve-end organs, is not scientifically admissible.

The apparent resting time may, perhaps, be considered as time during which these molecular processes are being transmitted along the excitable tissue very slowly. The blocking of the excitatory process in cardiac tissue by pressure, &c., is a case in point. The Torpedo presents us with a similar phenomenon, and this in spite of the fact that, once developed, the response of the electrical organ is far the

largest, and therefore the most easily appreciated, vital electromotive change which has up to this time been examined by the rheotome method. The extraordinary prolongation of the delay by cold, although apparently connected with the condition of the nerve endings, may, however, be at least partially accounted for by the increased time of transmission down the nerve branches contained in the organ itself. It is hoped that an early opportunity will afford means for ascertaining to what extent this is the case.

Experiments upon the Character and Duration of the Response.

For information on these points the experiments had to be of such a character that the results should indicate the extent of the electromotive change as appreciated by the galvanometer at successive periods after excitation, and it was found that the most practicable plan consisted in massing together all the changes which occurred during a period of $\frac{1}{100}$ ". The galvanometer circuit was closed for '01" at periods of '01", '02", '03", &c., after excitation of the nerve.

The results of such observations show that when the temperature of the organ is not below 10° C. the electromotive change develops with such rapidity that it reaches its maximum in less than one-hundredth of a second from the time at which it first becomes appreciable. If the change is expressed by a curve, the curve must thus be represented as rising very steeply from start to summit. The effect is always declining in the second and third hundredth of a second, and declines more slowly than it rose; but the rate of decline varies in different preparations, and thus the duration of the appreciable effect must vary. There is so marked a difference in this respect that, whereas in some cases no further change could be appreciated in the fourth hundredth of a second, in others a change was still perceived thirty seconds after the commencement of the response. This prolonged decline was, however, of such a character that it could be distinguished from the main decline of the organ response, and may be termed an "after-effect." Temperature, strength of response, and vigour and size of animal influence the above features of the response.

The influence of temperature is shown by the following experiment upon a nerve-organ preparation from a large Torpedo, 42 cm. \times 31 cm., the response of which did not show the prolonged "after-effect." It was placed upon the apparatus previously described; the total response to excitation with Galv. $\frac{1}{100}$ was + 7.0 scale.

The quickening of the response as the temperature rose rendered it necessary to shorten the time of closure of the galvanometer circuit, which was thus fixed at '005".

| Time of galvanometer closure :— | ·005"—·001" | ·01"—·015" | ·015"—·02" | ·02"—·025" | ·025"—·03" | ·03"—·035" | ·035"—·04" |
|----------------------------------|-------------|----------------------------|---------------------------|----------------------------|----------------------------|---------------------------|---------------------------|
| Temperature of organ 5° C. | .. | .. | .. | + 100 G. $\frac{1}{10}$ | + 165 G. $\frac{1}{10}$ | + 80 G. $\frac{1}{10}$ | + 10 G. $\frac{1}{10}$ |
| 20° C. | .. | + 130 G. $\frac{1}{10}$ | + 40 G. $\frac{1}{10}$ | .. | .. | .. | .. |
| 10° C. | .. | .. | + 100 | + 170 G. $\frac{1}{10}$ | + 40 G. $\frac{1}{10}$ | + 5 G. $\frac{1}{10}$ | .. |
| Interval of twenty-five minutes. | | | | | | | |
| 8° C. | .. | .. | + 240 | + ∞ | + 320 | + 30 | + 5 |
| 20° C. | .. | + ∞ | + 300 | + 70 | + trace | .. | .. |

In all cases the maximal intensity of effect is reached in '01" from its commencement, but at a high temperature this maximum is reached in the first half of this time, viz., '005"; at the low temperature it is reached in the second half of this time. The response is thus more suddenly developed at the higher temperature. This shortens the duration of the effect in the two cases, the decline of the effect not being so markedly affected as the rise is.

In the following experiment the minimal is compared with a maximal response of same organ. Small Torpedo, 13 cm. × 9 cm. Organ led off *in situ*. Induction apparatus with 2 Groves in primary coil (coil without core). Right side and left side used. Nerves excited *in situ*.

| Galvanometer closure :— | ·005"—·015" | ·015"—·025" | ·025"—·035" | ·035"—·045" | ·045"—·055" |
|---|-------------|-------------|-------------|-------------|-------------|
| Galv. $\frac{1}{10}$ Secondary coil 0 centims. . . | .. | + 370 | + 120 | + 25 | + trace |
| " 7 " . . | .. | + 60 | + 20 | 0 | .. |
| Left organ used. 4 Groves in primary circuit of coil. | | | | | |
| Secondary coil 15 centims. . | .. | + 85 | + 6 | 0 | .. |
| " 0 " . | .. | + ∞ | + 200 | + 50 | + trace |
| " 15 " . | .. | + 100 | + 18 | + trace | .. |

The minimal differs from the larger response more in its duration than in the rate of its development. Expressed as a curve, it would represent the cut-off summit of the large response. There are sometimes considerable differences in this respect between the duration of the responses of the two organs of the same fish, and the larger response is always the longer one. Thus the left organ of a small Torpedo gave a total response of + 100 G. $\frac{1}{10}$. Its character and duration are expressed by the following readings :—

| | Closure. | Deflection. |
|--------------------------------|----------|----------------------------------|
| Galvanometer closure | 014-016 | Galvanometer without shunt. 0 |
| " " | 016-018 | + 105; + 115 |
| " " | 018-2 | + 308; + 310 |
| " " | 02-022 | + 360 |
| " " | 022-024 | + 95 |
| " " | 024-026 | + 90 |
| " " | 026-03 | + trace |
| " " | 03 on | 0 |

The right organ of the animal gave a response of + 50 with G. $\frac{1}{100}$, and the decline of this response is much slower.

| | Closure. | Deflection. |
|--------------------------------|----------|--------------------------|
| Galvanometer closure | 014-016 | G. $\frac{1}{10}$. 0 |
| " " | 016-018 | + 95 |
| " " | 018-02 | + 380 |
| " " | 02-03 | + 220 |
| " " | 03-04 | + 15 |
| " " | 04-05 | + 10 |
| " " | 05-06 | .. |

Even a large Torpedo may give a response of short duration, but the response in such cases is always small in amount, and is, in fact, minimal.

Apart from the necessary effect of the vigour of the organ, there is that of the size of the fish, and so of the columns. The larger the fish, other things being equal, the longer the duration of what must be considered as the true response. This is shown by the results embodied in the annexed Table, in which the length of the columns of the organ is given.

l = length of column. r = total response.

| Time of galvanometer closure: | 01"-018" | 018"-02" | 02"-025" | 025"-03" | 03"-035" | 035"-04" | 04"-045" | 045"-05" |
|------------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|
| Small Torpedo, $l = 12$ mm. | + 12 | + 492 | + 400 | + 45 | + 20 | + 20 | + 4 | .. |
| G. $\frac{1}{10}$. | 0 | + 400 | + 470 | + 60 | + 10 | + trace | .. | .. |
| Ditto. G. $\frac{1}{10}$ | 0 | + 400 | + 470 | + 60 | + 10 | + trace | .. | .. |

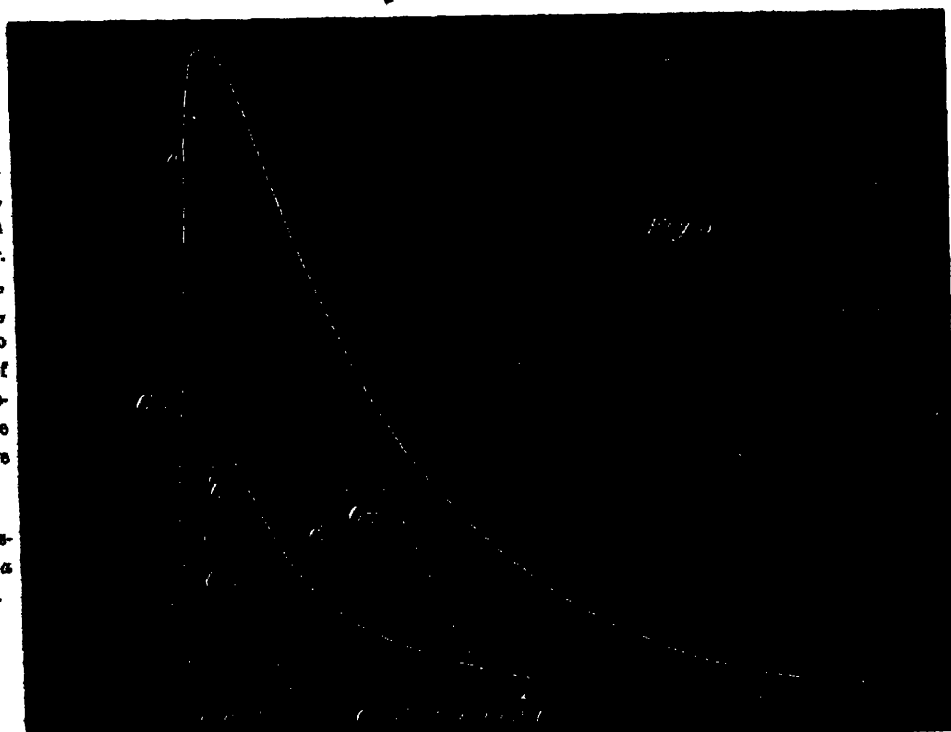
| Closure:— | ·01"—·02" | ·02"—·03" | ·03"—·04" | ·04"—·05" | ·05"—·06" | ·06"—·07" | |
|---------------------------------------|---|--|--|---|---|---|--------------------------|
| Medium-sized Torpedo, $l = 25$ mm. | $\left\{ \begin{array}{l} + \infty \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 620 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 190 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 16 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 5 \\ G. \frac{1}{100} \end{array} \right.$ | | . |
| " | $\left\{ \begin{array}{l} + 185 \\ G. \frac{1}{100} \end{array} \right.$ | | | | | | |
| " | $\left\{ \begin{array}{l} + \infty \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 740 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 155 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 35 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 4 \\ G. \frac{1}{100} \end{array} \right.$ | | |
| " | $\left\{ \begin{array}{l} + 105 \\ G. \frac{1}{100} \end{array} \right.$ | | | | | | |
| " | $\left\{ \begin{array}{l} + \infty \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 465 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 22 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 5 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + \text{trace} \\ G. \frac{1}{100} \end{array} \right.$ | | |
| " | $\left\{ \begin{array}{l} + 155 \\ G. \frac{1}{100} \end{array} \right.$ | | | | | | |
| Large Torpedo, $l = 53$ mm. | $\left\{ \begin{array}{l} + 700 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 450 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 120 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 23 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 7 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 13 \\ G. \frac{1}{100} \end{array} \right.$ | followed by after-effect |
| " | $\left\{ \begin{array}{l} + 510 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 305 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 165 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 60 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 25 \\ G. \frac{1}{100} \end{array} \right.$ | $\left\{ \begin{array}{l} + 15 \\ G. \frac{1}{100} \end{array} \right.$ | " |

The characters of the vigorous response are given by the above readings; and by taking the deflections as ordinates, and the times of closure as abscissæ, a curve may be

Fig. 3.

The numbers 100, 200, 300, &c., indicate scale of Galvanometer. The numbers 10, 20, 30, 40, &c., correspond to thousandths of seconds; 0 representing the moment of nerve excitation.

The transmission time in a and $b = \frac{1}{1000}$ sec.



drawn which will indicate the response. Such curves are given in fig. 3, and show the rapid rise and slower decline of the electromotive change. In the figure the curve a is that of a preparation made from a large vigorous Torpedo, b is that

of one from a medium-sized vigorous fish. The curves *c* and *d* are short curves representing the change in the organ of a large Torpedo, *c* being that of the organ at 20° C., and *d* that of the organ at 5° C. In the curve *a* the ordinates represent the readings of the galvanometer with the $\frac{1}{100}$ shunt, and the curve corresponds in duration to the duration of the effect as ascertained by MAREY.

In reality, however, the effect is prolonged far beyond seven hundredths of a second. This prolongation was apparently not capable of affecting MAREY's "physiological" galvanoscope, viz., the muscle-nerve preparation; its existence is, however, easily ascertained by the galvanometric method when the galvanometer is used without the shunt, and, owing to its slow subsidence, it produces well-marked galvanometric deflections. The large response has a long tail, if the expression may be used. It is termed here the "after-effect" of the response.

The after-effect of the response.—The fast-moving rheotome previously used was not adapted to the examination of the after-effect, since the range of the instrument did not extend beyond '08". The rheotome was therefore now used for giving an induction shock of constant intensity and duration for purposes of excitation, and the key K_2 was placed so as to be broken '06" after the exciting key K_1 . For K_3 there was substituted a key which could be closed quickly by the hand, this closure being effected at various intervals after the release of the traveller of the rheotome. Successive experiments were now made, first with the galvanometer circuit closed, but short-circuited by K_2 ; the deflection obtained thus indicated the effect which followed the excitation from '06" onwards. The galvanometer circuit was then closed 1" after the release of the rheotome, and the resulting deflection observed. The difference between the two readings was a measure of the amount of the slowly subsiding change during the period '06" to 1". The same plan was adopted with a closure 2", 3", 4", &c., after excitation.

As the after-effect following a response is a new feature of the excitatory change, it will be advisable to describe an experiment with some detail.

A block of organ was cut from a large vigorous Torpedo; the block measured 53 millims. in the direction of the columns, and was 35 millims. in width and 30 millims. in thickness. Its cut surface showed four injured columns, and, consequently, it contained twelve uninjured columns, all supplied by branches of the second electrical nerve. This was excited 20 millims. from its point of entry into the organ by the break of 2 Groves in the primary circuit of the induction apparatus at intervals of three minutes. The organ-current obtained by leading off the skin-covered ends of the columns amounted to + 0.125 R. It subsided rapidly, and then slowly, but sank after each excitation, and was compensated. This fall is given in terms of the galvanometer scale, it having been ascertained that a deflection of 130 scale corresponded to a difference of potential = 0.003 R. The fall is, of course, opposed to the after-effect. The galvanometer was used without its shunt.

| | | |
|--|-----------|--------------------|
| Galvanometer closed $\frac{1}{10}$ " after excitation | + 650 | } (.06"-1") 350 |
| Fall of organ-current, which subsided slowly, until in three minutes it sank 150 scale. It was then compensated. | | |
| Galvanometer closed 1" after excitation | . . + 300 | } (1"-2") 90 |
| Fall of organ-current as before = 80 scale. | | |
| Galvanometer closed 2" after excitation | . . + 210 | } (2"-3") 45 |
| Fall of organ-current = 75 scale. | | |
| Galvanometer closed 3" after excitation | . . + 165 | } (3"-4") 45 |
| Fall of organ-current = 75 scale. | | |
| Galvanometer closed 4" after excitation | . . + 120 | |

Experiment repeated:—

| | | |
|---|-----------|--------------------|
| Galvanometer closed $\frac{1}{10}$ " after excitation | + 640 | } (.06"-1") 400 |
| Fall of organ-current = 60 scale. | | |
| Galvanometer closed 1" after excitation | . . + 250 | } (1"-2") 60 |
| Fall of organ-current = 45 scale. | | |
| Galvanometer closed 2" after excitation | . . + 190 | } (2"-3") 35 |
| Fall of organ-current = 35 scale. | | |
| Galvanometer closed 3" after excitation | . . + 155 | |
| Fall of organ-current = 35 scale. | | |

It was evidently necessary to extend the observations far beyond the limit of 3", and this was now done in a similar preparation made from the organ of the other side of the same large Torpedo. In this the organ-current was very steady, the preparation having been made an hour before, and being first used for a rheotome experiment on the duration of the first part of the response. It amounted to .01 R., and was observed to sink after each excitation; the decline, being opposed to the after-effect, was only indicated after the return of the needle to zero, which occurred in about half a minute, by a slow movement of the needle in the opposite direction, amounting in three minutes to 75 scale.

| | | |
|---|---------------------------|--------------------|
| Galvanometer closed .06" after excitation | + ∞ | } (.06"-1") 450 |
| | (+120 G. $\frac{1}{10}$) | |
| " " 1" " " | + 750 | } (1"-2") 175 |
| " " 2" " " | + 575 | |
| " " 3" " " | + 450 | } (2"-3") 125 |
| " " 4" " " | + 365 | |
| " " 5" " " | + 270 | } (3"-4") 85 |
| " " 10" " " | + 205 | |
| " " 15" " " | + 160 | } (4"-5") 95 |
| " " 20" " " | + 135 | |
| " " 25" " " | + 100 | } (5"-10") 65 |
| " " 30' " " | + 75 | |
| | | } (10"-15") 45 |
| | | |
| | | } (15"-20") 25 |
| | | |
| | | } (20"-25") 85 |
| | | |
| | | } (25"-30") 25 |
| | | |

The difference between the successive readings is given in the right-hand column, from which it is seen that the after-effect sinks much more rapidly during the first 5" than subsequently, and that with regard to this first five seconds its fall is most rapid in the first two seconds.

The great prolongation of the after-effect allowed the employment of another method of reading, which had been used with good results in the investigation of prolonged polarisation after-effects. The "falling time" of the galvanometer needle was known to be fifteen seconds. The galvanometer was, therefore, closed for fifteen seconds, and the closure followed by a break of the circuit for the same time, this by a re-closure, and so on.

The deflections obtained are strictly comparable, each being procured with the galvanometer needle at zero, and any instrumental error thus obviated.

| | | |
|-----------------------------|------------------|---------------------------|
| Galvanometer circuit closed | 1"-15". | Effect + 650 |
| " | " broken 15"-30" | " .. + 130 |
| " | " closed 30"-45" | " + 60 |
| " | " broken 45"-60" | " .. + 5 |
| " | " closed 60"-75" | " + trace, followed by —. |

The two gaps 15"-30" and 45"-60" were filled up by repeating the experiment, reversing the times of closure and of break.

Another experiment on the same subject was suggested by the applicability of this method—an experiment in which the nerve should be excited mechanically.

Such mechanical excitation had been tried with the aid of TIGERSTEDT'S excitator (32), modified for this special research, but without any good result—the low excitability of the nerve being a bar to the use of this method, as only feeble and uncertain organ responses followed excitation of this character. It had, however, been noticed that section of the nerve always produced a large response of the organ. The investigation of the after-effect following the response evoked by section of the nerve was therefore quite practicable. In order to follow the change in the after-effect, the sensibility of the galvanometer was decreased by raising the control magnet, so that the falling time of the needle should be ten instead of fifteen seconds.

With the galvanometer circuit closed, the nerve of a nerve-organ preparation from a large vigorous Torpedo was divided with sharp scissors 35 millims. from the organ; a very large response followed the section, the deflection amounting to + 750 G. $\frac{1}{100}$.

On comparing this with the fullest response to electrical excitation which could be obtained in the same preparation, it was found to be of about equal amount, the latter giving + 670 G. $\frac{1}{100}$.

The galvanometer circuit was now closed and opened alternately at stated intervals after section of the nerve, and the prolonged after-effect thus observed:—

| | | |
|------------------------|-------------------------|-------|
| Circuit closed | 5" to 15" after section | + 440 |
| „ broken | 15" 25" „ „ | .. |
| „ closed | 25" 35" „ „ | + 230 |
| „ broken | 35" 45" „ „ | .. |
| „ closed | 45" 55" „ „ | + 160 |
| „ broken | 55" 65" „ „ | .. |
| „ closed | 65" 75" „ „ | + 110 |
| „ broken | 75" 85" „ „ | .. |
| „ closed | 85" 95" „ „ | + 65 |
| „ broken | 95" 105" „ „ | .. |
| „ closed | 105" 115" „ „ | + 20 |

The prolongation of the after-effect, or "excitation remainder," is most marked in this experiment. It will be noticed that it is always present when the response is such as to reach a great maximum of intensity, and this suggests the inquiry whether a similar change cannot be brought about by the passage through the tissue of an intense current of short duration. The experiments of DU BOIS-REYMOND previously referred to show that such is the case. To this subject we now proceed.

III. *The Electromotive Changes following the Passage of Electrical Currents through the Organ.*

The term "secondary" electromotive is applied by DU BOIS-REYMOND (33) to the changes produced in excitable tissues by the passage through these of electrical currents. The work of DU BOIS-REYMOND, followed by that of HERMANN (34), HERING (35), and BIEDERMANN (36), has shown that, in addition to the ordinary phenomena of electrolytic polarisation, there are special electromotive changes which are intimately connected with the vitality of the tissues experimented upon. The main fact may be broadly stated thus: the passage of a galvanic current of considerable intensity, and of short duration, through a tract of muscle or nerve, is followed not only by the electromotive changes accompanying ordinary polarisation, but by a prolonged electromotive change which presents the characteristics of being the electrical index of a prolonged excitation. This is not, strictly speaking, the view which DU BOIS-REYMOND took as to the nature of his discovery. According to the upholders of the molecular hypothesis, the passage of a galvanic current is followed by two sorts of polarisation—the ordinary negative polarisation, and a special positive polarisation, this last being an alteration in the condition of the vital electromotive molecules. It is simpler to call the latter effect an excitatory effect, and it will be thus designated here. The earlier work of DU BOIS-REYMOND has been succeeded by the publication of the results of similar experiments carried out upon the electrical organ of the Torpedo (37), which show that here, too, the passage of an intense current of short duration is followed by electromotive changes of two kinds, those due to electrolytic polarisation, and special phenomena, due, probably, to excitation of the tissue. The

latter reveal themselves as prolonged changes, in which the dorsal surfaces of the columns are galvanometrically positive to the ventral.

A polarising current directed through the tissue in the direction of that of the organ response is the most effectual for the production of the change in question. Such a polarising current has been termed by DU BOIS-REYMOND "homodromous"; it will be denoted in this work by the sign (+), since the cathode of the polarising circuit is on the dorsal surface of the columns. A current oppositely directed through the organ columns will be denoted by the sign (-).

If the phenomena of electrolytic polarisation, and these only, are present in the led-through tract, then a (+) polarising current will be followed by a (-) after-effect, a (-) polarising current by a (+) after-effect, the signs referring in all cases to the condition of the dorsal electrode.

Such is the case in the following example, which is given to show the polarisable character of the tissue.

A strip of organ, consisting of several entire columns, having been prepared from a small Torpedo, and measuring 13 mm. in length and 2 mm. in thickness, was placed upon a glass plate and led off as usual from the dorsal and ventral ends. Upon the kaolin plugs of the leading-off electrodes were placed the kaolin plugs of another pair of non-polarisable electrodes, which served to lead the polarising current through the tissue. A paraffin switch of the kind used by HERMANN was introduced into the galvanometer and polarising circuits. By its movement the galvanometer circuit was broken, and the polarising circuit closed about $\frac{1}{10}$ " afterwards; the return of the switch broke the polarising and then re-closed the galvanometer circuit. The movement of the switch was effected in the one direction by the hand, in the other by an elastic spring. The sensibility of the galvanometer was the same as in the early experiments, and the falling time of the needle was fifteen seconds. The effect was therefore accurately indicated by reading the amount of the deflection every fifteen seconds.

| Polarising current. | 15" readings. | 15" readings. | 15" readings. |
|--|--|---|--|
| 4 Groves, duration $\frac{1}{2}$ ", direction (+), gal- vanometer $\frac{1}{10}$ | - 265 - 170 - 130 - 100 - 70 - 50 - 30 | - 230 - 110 - 50 - 30 - 15 | - 230 - 110 - 40 - 10 |
| 4 Groves, duration 1", direction (+), gal- vanometer $\frac{1}{10}$ | - 360 - 200 - 110 - 70 - 45 - 30 | - 380 - 200 - 100 - 60 - 40 - 20 | - 400 - 200 - 110 - 60 - 40 - 20 |
| 4 Groves, direction (-), galvanometer $\frac{1}{10}$ | duration $\frac{1}{2}$ " + 170 + 70 + 45 | duration 2" + 260 + 120 + 60 + 40 | duration 3" + ∞ + 350 + 190 + 140 + 110 + 100 + 85 + 80 |

Besides showing the susceptibility of the tissue to polarisation, and the subsidence of the effect, the experiment shows that the (+) polarising current is more effectual than the (-) for the production of the polarisation after-effect. The polarising current was increased to 6 Groves without altering the result as regards the direction of the after-effect. When, however, the current of 6 Groves was led through narrow strips of fresh tissue cut from the organs of vigorous fish, a different result was obtained. The passage of the (+) current was now followed by a (+) after-effect; on repeating the experiment, the effect soon disappeared, and only the (-) polarisation after-effect was observed. Two examples may be given.

Strips of organ, 22 mm. long and 3 mm. thick, were cut from the organ of a medium-sized fish. Organ-current, + '0062 R.

| Polarising current. | Duration. | | | | |
|---|-----------------|-----------------|-----------------|-----------------|-----------------|
| | $\frac{1}{2}$ " | $\frac{1}{2}$ " | $\frac{1}{2}$ " | $\frac{1}{2}$ " | $\frac{1}{2}$ " |
| 6 Groves, galvanometer $\frac{1}{10}$ (+) | - 70 | + 190 | - 65 | - 110 | - 190 |

| Polarising current. | Duration $\frac{1}{2}$ " |
|---------------------------------|--------------------------|
| Galvanometer $\frac{1}{10}$ (-) | + 135 |

Another strip, 30 mm. long and 4 mm. in thickness.

| Polarising current. | Duration. | | | | |
|---|-----------------|-----------------|-----------------|--------------|-------------------|
| | $\frac{1}{4}$ " | $\frac{1}{2}$ " | $\frac{3}{4}$ " | 1" | 1 $\frac{1}{2}$ " |
| 6 Groves (+), galvanometer $\frac{1}{10}$ | + 150 | - 40 | + 110 | + 30 - 20 | - 120 |

The strip was now pared down in the direction of the columns, so as to reduce it to a long strip only 2 mm. in thickness.

| Polarising current. | Duration | | | |
|---|-----------------|-----------------|-----------------|-------|
| | $\frac{1}{4}$ " | $\frac{1}{2}$ " | $\frac{3}{4}$ " | 1" |
| 6 Groves (+), galvanometer $\frac{1}{10}$ | + ∞ | + 400 | - 100 | - 160 |

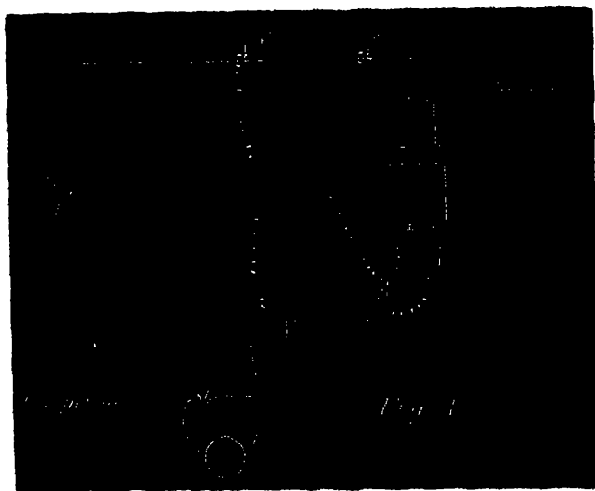
| Polarising current. | Very short. | Very short. |
|---|-------------|-------------|
| 6 Groves (+), galvanometer $\frac{1}{10}$ | + 220 | - 50 |

In all these cases further experiments with (+) currents gave only (-) after-effects.

It is obvious that, whereas the (+) after-effect obtained with (-) currents was only the ordinary change of electrolytic polarisation, the (+) after-effect following (+) currents was something of a different nature, and was the effect noticed by DU BOIS-REYMOND (38). In order to obtain it with more distinctness, it was necessary to use stronger polarising currents, but unfortunately more than 6 Groves were not obtainable. In considering the question of its production, and especially the characteristics of the current which produced it, the essential feature is seen to be the necessity of using polarising currents of short duration and great intensity. The reason is probably that with these the ordinary phenomena of electrolytic polarisation are not developed in an overwhelming degree. If the polarising currents are long, the polarisation after-effects are so marked as to swamp every other electromotive change. This being the case, the use of strong induction currents at once suggested itself. The experiments of DU BOIS-REYMOND with repeated induction currents (39) gave every hope of success, and, as the ordinary electrolytic phenomena would be reduced to a minimum, the confusion between polarisation and other changes would be largely avoided.

The after-effect of the passage of induction shocks.—In order to carry out the experiment, the previous method was modified as follows :—The spring myograph was substituted for the paraffin switch. The key K_1 of the instrument was placed in the primary circuit of the induction apparatus. The galvanometer circuit was arranged as in fig. 4, the secondary coil being directly in the circuit, the induction shock being

Fig. 4



short-circuited by the key K_2 so as to prevent any disturbance of the instrument ; to render this effectual, a resistance of 10,000 ohms was introduced between the key and the galvanometer. It was ascertained that with K_2 placed so as to be opened '003" after K_1 no appreciable effect was observed by the passage of a strong induction shock through the electrodes. By this method an induction shock could be led through the tissue, and the galvanometer would respond to all electromotive changes which followed that at an interval of '003". The same electrodes must obviously be used both for leading in and leading off. It was ascertained by experiment that any after-effect due to polarisation in these was extremely small.

From the organ of a medium-sized fish a strip was cut measuring 16 mm. in length, 7 mm. in width, and 2 mm. in thickness. The induction apparatus was used with 3 Groves in the primary coil ; the secondary coil stood at 5 cm., organ-current + '006 R.

| Direction of induction shock through tissue (—) | | | | After effect | |
|---|---|---|---|----------------------|---------|
| | | | | Galv. $\frac{1}{10}$ | + 150 |
| " | " | " | " | (+) | " + 650 |
| " | " | " | " | (—) | " + 180 |
| " | " | " | " | (+) | " + 730 |

This (+) after-effect thus occurred with both (+) and (—) currents, but the former were far the most effectual for its production. Since the polarisation phenomena are by this method reduced to a minimum, the after-effects here observed are due to the particular change which the tissue shows apart from those. This change was noticed

to be prolonged in character, like the after-effect which follows the nerve-organ response. Its duration and rate of subsidence were investigated in the same way as the latter had been, by closing the galvanometer circuit for 15" after excitation, opening it for 15", re-closing it for 15", &c. The readings of the deflections obtained in four experiments were as follows :—

| Direction of induction shock coil 5 cm. (+) Galv. $\frac{1}{10}$ | Closure | Experiment 1. | Experiment 2. |
|---|---|--|--|
| | ·003"-15" 30"-45" 60"-75" 90"-105" | + ∞, sinking to + 500 + 500 + 98 + 58 | + ∞, sinking to + 400 + 110 + 62 + 40 |
| Direction of induction shock coil 7 cm. Galv. $\frac{1}{10}$ | ·003"-15" 30"-45" 50"-75" 90"-105" | (+) Experiment 3. + 510, sinking to + 260 + 85 + 52 + 33 | (-) Experiment 4. + 225, sinking to + 170 + 68 + 40 + 25 |

The induction shock is thus followed by a prolonged electromotive change which subsides at first rapidly and then more slowly, thus resembling the after-effect of the response. It differs from this as regards its duration, as it lasts somewhat longer. As it can be produced by induction currents in either direction, (+) or (—), it can only be an excitatory change. It is always of the character which the excitatory changes of the organ follow, the dorsal surface of the organ becoming (+). It is remarkable that it is more effectually produced by (+) than by (—) induction currents. This does not prevent our regarding it as an excitation phenomenon, for the nerve itself is more readily excited by descending than by ascending currents, and a (+) current through the organ may play the part of a descending one for the majority of nerves and nerve endings which lie in its path. If it is an excitation phenomenon, the organ must behave like the nerve with respect to increasing strength of stimulus. That this is so the following experiment shows, the numbers indicating readings of the position of the light on the scale made at intervals of 15" after the maximum deflection had been reached. Strip of organ, 36 mm. long, 5 mm. wide.

